Modeling the vector-borne disease transmission potential in northern Europe with a special emphasis on microclimatic temperature

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Modeling the vector-borne disease transmission potential in northern Europe with a special emphasis on microclimatic temperature

PhD Thesis

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Front cover: Maps of 22,004 Danish cattle farms showing a 17 year average of the extrinsic incubation period (EIP) of Schmallenberg virus. The maps compare EIP calculated using temperatures from the Danish Meteorological Institute (top map) and estimated microclimatic temperature (bottom map). The image of *Culicoides* is used with the permission of USDA (www.usda.gov) for non-commercial purpose. Image of Danish Meteorological Institute's (DMI) weather station located at Borris, Ringkøbing-Skjern municipality is used with permission from DMI.

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Preface and acknowledgements

When I was selected for this PhD position back in November 2014, I shared the news with my colleagues and supervisor at ICDDR,B (International Centre for Diarrhoeal Disease Research, Bangladesh). One of my colleagues asked me “are there any vector-borne diseases in Denmark?”. I could answer him only one name - “bluetongue virus”. After 3.5 years of research in Denmark, I now know some more names of vector-borne diseases in Denmark and I know how likely the temperature of Denmark is for a potential vector-borne disease outbreak. My PhD is all about modeling vector-borne disease transmission potential in northern Europe with a special emphasis on microclimatic temperature. We recorded microclimatic temperature from six different habitats, compared it with standard meteorological temperature and quantified their impacts on the extrinsic incubation period of six pathogens (bluetongue, Schmallenberg, dengue, and West Nile virus, and Malaria and Dirofilaria parasites). We also developed models that can predict the microclimatic temperature of these habitats based on meteorological parameters readily available from any meteorological institute. We have modeled the transmission potentials of bluetongue virus in Denmark and *Setaria tundra* in Finland using both microclimatic and meteorological temperature. This PhD project was funded by the Danish Food and Veterinary Administration, EMIDA ERA-NET supported project ‘VICE Risk-based Surveillance for Vector-Borne Diseases’, EurNegVec COST Action TD1303 framework.

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In 2014, my supervisor met two Finnish researchers at the EurNegVec conference. That meeting gave me the opportunity to work on *S. tundra* and to visit Finland during my PhD. Sauli Laaksonen’s knowledge of *S. tundra* is unparalleled. It was an amazing opportunity to work with the data Sauli had collected over 12 years, to visit reindeer rounds-up (animal gathering for deworming and slaughtering) and hunting cottage. Antti

Oksanen's information about reindeer husbandry and critical evaluation of my graphs during my stay in the Finnish Food Safety Authority-EVIRA were invaluable. I am very thankful to both of you and your family. I would like to thanks the Finnish Meteorological Institute for sharing the temperature data of the period 1979-2015.

We are grateful to Danish Meteorological Institute (DMI) and thankful to Jens Havskov Sørensen, Chief Consultant of R&D department of DMI for all the cooperation related with data sharing, interpretation and critical input in the manuscript. I would like to thank Birgit Kristensen for helping me set up the initial temperature data loggers at Strødam. I would also like to thank David Bille Byriel for all the help during the entire fieldwork season in 2015. Cecilie Grønlund Clausen recorded my temperature logger's data when I was sick – I am so grateful to you Cecilie.

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My father would have been so proud of me completing my PhD thesis and I wish he were still with us today. I am grateful to my mom for all the support. I would like to convey a very special thanks to my amazing wife Tania and two daughters Aarha Imtinan and Aairah Ilmeeyat. My wife took on the most of the child-rearing responsibilities while I undertook this research. You three make me the happiest person in the world and I look forward to spending additional time with you now. I would also like to acknowledge my extended family especially my two elder brothers, Abdul Halim and Abdul Mannan without who I would not be where I am today.

Finally, I would like to acknowledge the Almighty creator, the omnipotent and supreme authority of this universe for allowing me to finish the thesis in good health.

Najmul Haider
DTU, Lyngby, Denmark
13 August 2018

Summary

Denmark experienced bluetongue outbreaks in 2007 and 2008 even though the Danish climate was previously considered to be too cold for the transmission of a number of vector-borne diseases (VBD), including the disease caused by bluetongue virus (BTV). BTV causes weight loss, abortion, reduced milk yield and ultimately death in ruminants (cattle and sheep), and is responsible for restricted trade of animals and animal products internationally. Schmallenberg is an emerging *Culicoides*-borne viral disease that can affect cattle, sheep and goats, and causes abortion and stillbirth with congenital malformation. Schmallenberg virus was detected in Denmark in 2012. Epidemiological modeling plays an important role in policy making and strategies for the prevention and control of VBD. Deciding what approach to take requires models able to generate accurate predictions, which in turn relies on an accurate knowledge of the natural lifecycle of vectors, including the daily survival probability, biting rate, extrinsic incubation period (EIP) of the virus, and vector competence. All these parameters are dependent on the temperature to which the insect vectors are exposed. Mathematical models used to quantify VBD transmission parameters are mostly driven by temperatures recorded by meteorological institutes. Insects are poikilothermic and they experience temperatures at a very local level, down to a scale of meters or even centimeters. Models that use meteorological temperatures do not take into account the temperature to which the insects are exposed in the microhabitat. Weather stations may be located as far as 50–100 km from the insect resting sites, and the lack of data from microclimatic environments hinders the use of microclimatic temperatures in disease modeling.

The aim of this thesis was to improve our understanding of the transmission potential of VBD in northern Europe. We conducted four studies and systematically studied the microclimatic temperature of potential insect habitats at different heights for an entire warm season (May to October). We also systematically studied the role of microclimatic temperature on VBD transmission potential, as well as the variation and uncertainty associated with bluetongue virus transmission in Denmark. Finally, we studied the potential role of temperature on the occurrence of *Setaria tundra* outbreaks (a mosquito-borne filarioid disease) and on the intensity of worm transmission to reindeer in general.

In the first step, we recorded the hourly temperature in six microclimatic habitats (dry meadow, wet meadow, hedges, trees, and cattle and horse fields) in Strødam and Faxe in Denmark from May to October, 2015. We did this by setting temperature data loggers in triplicate and at 2 or 3 different heights in each habitat. We also collected information on the hourly meteorological temperature, solar radiation, wind speed, humidity, and precipitation from the Danish Meteorological Institute (DMI)'s weather stations in the same areas. We performed multiple linear regressions to predict the microclimatic temperature of different habitats based on the parameters collected from meteorological weather stations. Finally, we estimated the development time of six vector-borne pathogens (bluetongue, Schmallenberg virus, West Nile virus, Dirofilaria, malaria and dengue) that were either reported in Denmark or likely to be introduced to the country, by developing rate-summation models for each pathogen.

In the second step, we quantified the different land cover classes within a 500 m radius of all cattle farms in Denmark (N=22,004) using CORINE land cover, and regrouped them into four major land cover types: dry meadow, wet meadow, hedges, and forest. We then obtained the meteorological temperatures and other parameters (solar radiation, wind speed, humidity) near the farm between 2000 and 2016 from DMI. We calculated the hourly microclimatic temperatures of each farm based on their surrounding habitat types and meteorological parameters using our microclimatic temperature prediction models for those four major land cover types. We then modeled the daily EIP of Schmallenberg virus for each farm for the period 2000-2016 using both hourly DMI and hourly microclimatic temperatures, and calculated the mean EIP over the 17 years for each farm.

In the third step, we used the microclimatic temperature data for all Danish cattle farms in a mechanistic transmission model (biological process model) to estimate the daily number of infectious bites (IB) with BTV using *Culicoides* midge abundance data based on 1,453 light trap collections from 2007-2016. We used published equations for the EIP, daily vector survival rate, daily vector biting rate, and host-to-vector transmission rate, and quantified the variation and sensitivity of each parameter associated with daily IB estimates.

In the final step, we studied *S. tundra*, a mosquito-borne filarioid parasite, in northern Finland (Lapland). We developed a temperature-driven transmission model that was able to predict the potential number of *S. tundra* transmitted from an infectious reindeer.

In the first study, we found that microclimatic habitats were warmer during the day and cooler at night compared to the DMI recorded temperatures. The estimated EIP was shorter for five of the six microclimatic habitats compared to the estimates based on DMI temperatures for all pathogens studied. The microclimatic temperatures also predicted a longer season for virus development compared to DMI temperatures. Based on DMI data of hourly temperature, solar radiation, wind speed, rain and humidity, we were able to predict the microclimatic temperature of different habitats with an R^2 of 0.87-0.96.

In the second study, we found a large mean difference between the temperature of the warmest and coolest habitats within a 500m radius of a farm. On average, the difference was found to be 3.7°C (5^{th} and 95^{th} percentiles: 1.0°C to 7.8°C). The mean EIP of Schmallenberg virus (5^{th} and 95^{th} percentiles) for all cattle farms during spring, summer, and autumn were 23 (18–33), 14 (12–18) and 51 (48–55) days, respectively, assuming that *Culicoides* select resting sites randomly. These estimated EIP values were considerably shorter than those estimated using standard meteorological temperatures obtained from DMI for the same periods: 43 (39–52), 21 (17–24) and 57 (55–58) days, respectively. We observed a large area where farms with shorter EIP for Schmallenberg virus were grouped together. This area included southern Funen (and associated islands), Lolland, Falster, and southern Zealand.

In the third study, we estimated the maximum daily number of IB with BTV at 2,899, while the best-case scenario suggested transmission would not be possible in Denmark. The mean (10-90^{th} percentiles) daily number of IB was 2.2 (0-8.9) during the transmission period. Temperature and vector abundance were the most influential drivers in the estimates of the daily transmission potential. We found a large uncertainty associated with the daily insect survival rate and transmission rate from host to vector. The mean number of IB above two for the entire season indicates that bluetongue disease can become epidemic if introduced, especially when taking into consideration that an infected host remains infectious for approximately 3 weeks.

In the final study, our model showed that temperatures never allowed for transmission of more than a single generation of *S. tundra* each season in Finland. We found that larger outbreaks were recorded in the southern regions of the reindeer-herding areas compared to the central or northern parts of Lapland. In southern and central Lapland, our model predicted an increasing trend from 1979 to 2015 for both the duration of the effective transmission period of *S. tundra* and for the potential number of L3 *S. tundra* larvae being transmitted from an infectious reindeer.

The findings of this thesis will hopefully increase our understanding of the potential transmission of several VBD in northern Europe. Our studies consistently showed that microclimatic temperature has a significant impact on VBD in three ways: i) it decreases the pathogen development period (shorten length of EIP), ii) it prolongs the transmission season, and iii) it expands the geographical region of potential VBD transmission. The hourly microclimatic temperature data generated for all cattle farms in Denmark will remain a valued resource for studying these and other temperature-sensitive diseases. Our findings showed that BTV can spread rapidly if introduced to a farm. However, we also identified a large uncertainty associated with several parameters including the daily insect survival rate and host-to-vector transmission rate, which should be used with caution in BTV modeling. Temperature and vector abundance are two important drivers of BTV transmission. In Finland, the effective transmission period for *S. tundra* in reindeer was very short, but it increased over the period studied. Only one generation of *S. tundra* can be transmitted in one season among reindeer in Lapland, but increasing temperatures may facilitate a range expansion and increasing duration of the effective transmission period.

Sammendrag

Danmark oplevede udbrud af sygdommen bluetongue i 2007 og 2008, selvom det danske klima tidligere blev betragtet som for koldt for overførsel af en række vektorbårne sygdomme (VBS), herunder sygdommen forårsaget af bluetongue virus (BTV). BTV forårsager vægttab, abort, reduceret mælkeudbytte og i sidste ende død hos drøvtyggere (kvæg og får) og er ansvarlig for begrænsning i handel med dyr og animalske produkter internationalt. Schmallenberg er en Culicoides-båret virussygdom, der kan påvirke kvæg, får og geder og forårsager abort og dødfødsel med medfødte misdannelser. Schmallenberg-virus blev opdaget i Danmark i 2012. Epidemiologisk modellering spiller en vigtig rolle ved beslutningstagning og strategier til forebyggelse og kontrol af VBS. For at optimere disse beslutningstiltag, kræves modeller, der kan generere nøjagtige forudsigelser, baserede på nøjagtig viden om vektorernes naturlige livscyklus, herunder den daglige overlevelsessandsynlighed, bidrate, ekstrinsisk inkubationsperiode (EIP) af virusset og vektorkompetence. Alle disse parametre er afhængige af den temperatur, som insektvektorer er udsat for. Matematiske modeller, der bruges til at kvantificere VBS-transmissionsparametre, drives hovedsageligt af temperaturer registreret af meteorologiske institutter. Insekter er imidlertid poikilotermiske, og påvirkede af temperaturer på et meget lokalt plan, ned til en skala af meter eller endda centimeter. Modeller, der bruger meteorologiske temperaturer, tager ikke højde for temperaturen, som insekterne udsættes for i deres mikrohabitat. Vejrstationerne kan være placerede så langt som 50-100 km fra insekternes hvilepladser, og manglen på data fra mikroklimatiske miljøer forhindrer anvendelsen af mikroklimatiske temperaturer i sygdomsmodellerings.

Formålet med denne afhandling var at forbedre vores forståelse af VBS's transmissionspotentiale i Nordeuropa. Vi gennemførte fire undersøgelser og undersøgte systematisk mikroklimatiske temperaturer som potentielle insekthabitater ved forskellige højder for en hel varm sæson (maj til oktober). Vi undersøgte også systematisk mikroklimatiske temperaturers rolle på VBS-transmissionspotentialet, såvel som variation og usikkerhed forbundet med bluetongue-virusoverførsel i Danmark. Endelig vurderede vi temperaturens potentielle rolle for forekomsten af *Setaria tundra*-udbrud (en myggebåren filarioid sygdom) og på intensiteten af overførsel af orm til rensdyr generelt.

Først registrerede vi timetemperaturen i seks mikroklimatiske habitater (tør eng, våd eng, hække, træer og heste- og kvægmarker) i Strødam og Faxe i Danmark fra maj til oktober, 2015. Vi gjorde det ved at indstille temperatur dataloggere i triplikater og i 2 eller 3 forskellige højder i hvert habitat. Vi indsamlede også oplysninger om den timevise meteorologiske temperatur, solstråling, vindhastighed, fugtighed og nedbør fra Dansk Meteorologisk Instituts (DMI) vejrstationer i samme områder. Vi udførte lineære regressioner for at prædiktere mikroklimatiske temperaturer i forskellige levesteder baseret på parametre indsamlet fra meteorologiske vejrstationer. Endelig estimerede vi udviklingen af seks vektorbårne patogener (bluetongue, Schmallenberg-virus, West Nile-virus, dirofilaria, malaria og dengue), som enten er blevet rapporteret i Danmark eller potentielt kan blive introduceret til landet ved at udvikle rate-summationsmodeller for hvert patogen.

Efterfølgende kvantificerede vi de forskellige habitater inden for en 500 m radius af alle kvægbrug i Danmark (N = 22.004) ved hjælp af CORINE land cover og reklassificerede dem til følgende 4 habitater: tør eng, våd eng, hække og skov. På bagrund af DMIs meteorologiske temperaturer og andre parametre (solstråling, vindhastighed, fugtighed) nær de enkelte kvægbrug fra årene 2000 til 2016 fra DMI beregnede vi de timevise mikroklimatiske temperaturer på hvert kvægbrug baseret på deres omgivende habitattyper og meteorologiske parametre ved hjælp af vores mikroklimatiske temperaturforudsigelsesmodeller for de fire store habitater. Vi modellerede derefter den daglige EIP for Schmallenberg-virus for hvert kvægbrug i perioden 2000-2016 ved brug af både timevise DMI og mikroklimatiske temperaturer og beregnede den gennemsnitlige EIP over 17 år for hvert kvægbrug.

Dernæst brugte vi mikroklimatiske temperaturdata for alle danske kvægbrug i en mekanistisk transmissionsmodel (biologisk procesmodel) til at estimere det daglige antal infektiøse bid (IB) indeholdende BTV ved hjælp af *Culicoides* mittedata baseret på 1.453 lysfælde-indsamlinger fra 2007-2016. Vi anvendte offentliggjorte ligninger for EIP, daglig vektoroverlevelsesrate, daglig vektorbiderate og værts-til-vektor-transmissionshastighed til brug for kvantificering af variation og følsomhed for hver parameter forbundet med daglige IB-estimater.

I sidste trin studerede vi *S. tundra*, en myggebåren filarioid parasit, i det nordlige Finland (Lapland). Vi udviklede en temperaturdrevet transmissionsmodel, der var i stand til at forudsige det potentielle antal *S. tundra* transmitteret fra et infektiøst rensdyr.

I den første undersøgelse fandt vi, at mikroklimatiske habitater var varmere om dagen og køligere om natten end de af DMI registrerede temperaturer. Den estimerede EIP var kortere for fem af de seks mikroklimatiske habitater sammenlignet med estimaterne baseret på DMI's temperaturer for alle undersøgte patogener. De mikroklimatiske temperaturer prædikterede også en længere sæson for virusudvikling sammenlignet med DMI's temperaturer. Baseret på timedata fra DMI, timetemperatur, solstråling, vindhastighed, regn og fugtighed, kunne vi forudsige den mikroklimatiske temperatur i forskellige habitater med en R^2 på 0,87-0,96.

I den anden undersøgelse fandt vi en stor gennemsnitlig forskel mellem temperaturen på de varmeste og koldeste habitater inden for en radius af 500m fra kvægbrug. I gennemsnit viste forskellen sig at være 3,7° C (5 og 95 percentiler: 1,0 °C til 7,8 °C). Den gennemsnitlige EIP af Schmallenberg-virus (5 og 95 percentiler) for alle kvægbrug i forår, sommer og efterår var henholdsvis 23 (18-33), 14 (12-18) og 51 (48-55) dage, forudsat *Culicoides* vælger hvilesteder tilfældigt. Disse estimerede EIP-værdier var væsentligt kortere end de estimerede ved anvendelse af standard meteorologiske temperaturer fra DMI i samme perioder: Henholdsvis 43 (39-52), 21 (17-24) og 57 (55-58) dage. Vi observerede et stort område, hvor kvægbrug med kortere EIP for Schmallenberg-virus blev grupperet sammen. Dette område omfattede det sydlige Fyn (og tilhørende øer), Lolland, Falster og Sydsjælland.
I den tredje undersøgelse estimerede vi de maksimale daglige IB indeholdende BTV til værende 2.899 bid, mens transmission i det bedst mulige scenario ikke ville være muligt i Danmark. Det gennemsnitlige (10-90 percentiler) daglige antal IB var 2,2 (0-8,9) i transmissionsperioden. Vektortæthed og temperatur var de mest indflydelsesrige parametre i estimaterne af det daglige transmissionspotentiale. Vi fandt en stor usikkerhed forbundet med den daglige insekt-overlevelsesrate og transmissionshastighed fra vært til vektor. Det gennemsnitlige antal IB på over 2 for hele sæsonen indikerer, at bluetongue kan føre til en epidemi, hvis indført i landet, især når man tager i betragtning, at en inficeret vært forbliver infektiøs i ca. 3 uger.

I den sidste undersøgelse viste vores model, at temperaturer aldrig tillod overførsel af mere end en enkelt generation af *S. tundra* hver sæson i Finland. Vi fandt ud af, at større udbrud blev registreret i de sydlige regioner i rensdyrbesætningen i forhold til de centrale eller nordlige dele af Lapland. I det sydlige og centrale Lapland forudsagde vores model en stigende tendens fra 1979 til 2015 for både varigheden af den effektive overføringsperiode og det potentielle antal L3 *S. tundra* larver, der overføres fra et smitsomt rensdyr.

Resultaterne af denne afhandling vil forhåbentlig forbedre vores forståelse for den potentielle transmission af flere VB'er i Nordeuropa. Vores undersøgelser viste konsekvent, at mikroklimatiske temperaturer har en betydelig indvirkning på VBS på tre måder: i) det reducerer patogenudviklingsperioden (længden af EIP), ii) den forlænger transmissionssæsonen, og iii) den udvider den geografiske region for potentiel VBS transmission. De timevise mikroklimatiske temperaturdata genereret for alle kvægbrug i Danmark vil forblive en værdsat ressource til at studere disse og andre temperaturfølsomme sygdomme. Vores resultater viste, at BTV kan spredes hurtigt, hvis det introduceres til en gård. Vi identificerede dog også en stor usikkerhed forbundet med flere parametre, herunder den daglige insektoverlevelsesrate og værts-til-vektor-transmissionshastighed, som derfor bør anvendes med forsigtighed i BTV modellering. Temperatur og vektortæthed er 2 vigtige parametre, der driver BTV transmission. I Finland var den effektive transmissionsperiode for *S. tundra* i rensdyr meget kort, men den steg i den undersøgte periode. Kun én generation af *S. tundra* kn overføres i en sæson blandt rensdyr i Lapland, men stigende temperaturer kan føre til større udbredelse og en øget varighed af den effektive transmissionsperiode.

List of abbreviation

BT	Bluetongue
BTV	Bluetongue virus
CHR	Central husbandry register
CIMO	Commission for Instruments and Method of Observation
CORINE	Coordination of information on the environment
DMI	Danish Meteorological Institute
DTU	Technical University of Denmark
EIP	Extrinsic incubation period
EUR	Euro
FMI	Finnish Meteorological Institute
HIRLAM	High-resolution limited area model
IB	Infectious bites
M-K test	Mann-Kendall test
OIE	World Organization for Animal Health
QGIS	Quantum global information system
SAS	Statistical analysis software
SCH	Schmallenberg virus
USD	U.S. dollars
UTC	Coordinated universal time
VBD	Vector-borne disease(s)
VC	Vectorial capacity
WMO	World Meteorological Organization

Chapter 1: Introduction

1. Introduction

1. 1 Vector-borne disease

Vector-borne diseases (VBD) are the infections transmitted by infected arthropod vectors including mosquitoes, ticks, sand flies, fleas, and biting midges [1]. Historically, these vectors were responsible for plagues such as the "Black death" in fourteenth century Europe [2]. The "Black death" was caused by the bacterium *Yarsinia pestis*, which was carried by rodent fleas and killed 30-60% of the total population of Europe [2]. Currently, VBD account for more than 17% of all infectious diseases in the world and are responsible for more than 700,000 human deaths annually [1]. Dengue is the most common VBD with approximately 4 billion people at risk of contracting the disease and 96 million human cases per year [1]. Malaria is responsible for more than 400,000 human deaths annually around the world [1]. Chikungunya, Zika, Rift Valley fever, yellow fever, lymphatic filariasis, Japanese encephalitis, West Nile virus, Lyme disease, and tick-borne encephalitis are also common VBD in humans around the world [1]. A review study on emerging infectious diseases identified 22.8% of all disease as vector-borne [3].

A number of VBD, including Bluetongue virus (BTV), Schmallenberg virus (SCV), African horse sickness, leishmaniosis, dirofilariasis, *Setaria tundra*, West Nile virus, and Crimean-Congo haemorrhagic fever [4] are of particular importance in animals and birds due to their ability to cause morbidity and mortality as well as economic loss. BTV was first recognized in South Africa more than 100 years ago and has probably been present for as long as there has been sheep farming in the area. The first recognized outbreak of BTV in Europe occurred in Cyprus in 1943, and there was another in Portugal in 1956 [5]. The largest BTV outbreak hit Europe in 2006-2008, when BTV serotype 8 affected a number of central and northern European countries [5]. The current European outbreak of BTV-8 is believed to have caused greater economic damage than any previous single-serotype outbreak [5]. Due to its global significance, BTV is regarded as a notifiable disease by the World Organization of Animal Health (OIE) [6]. BTV is responsible for global losses of an estimated 3 billion USD per year [6]. In August 2011, an unknown disease causing fever, reduced milk yield in dairy cows and congenital malformation in calves [7] was observed in Germany and subsequently named Schmallenberg virus. Culicoides biting midges were later found to carry and transmit

Schmallenberg virus [8]. *Setaria tundra*, a mosquito-borne filarial parasite has been associated with three large outbreaks in Finnish reindeer [9]. The *S. tundra* outbreak in 1973 was associated with a high mortality rate as the Finnish reindeer population decreased from 140,000 to 98,000 [10]. The disease is associated with peritonitis, perihepatitis and poor body condition [9–11].

1. 2 Vector-borne disease in Denmark

The Danish climate was previously predicted to be too cold for the transmission of a number of vector-borne diseases including BTV [12], yet the country experienced a large BTV outbreak in 2007 and 2008 [13]. BTV causes death, weight loss, abortion, reduced milk yield of ruminants (cattle and sheep), and is responsible for restricted trade of animals and animal products internationally [6]. As a consequence, in 2008-2009, the Danish cattle industry spent more than 5 million EUR vaccinating cattle in order to prevent a widespread epidemic [14]. Schmallenberg is an emerging *Culicoides*-borne viral disease that can affect cattle, sheep and goats, and causes abortion and stillbirth with congenital malformation [7,15]. Schmallenberg virus was first detected in Denmark in 2012 [16].

Plasmodium vivax malaria was a common endemic disease in Denmark in the nineteenth century [17–19]. The south eastern parts of the country, especially Lolland-Falster and south Zealand were severely affected by Malaria [20]. During the second half of nineteenth century, malaria steadily declined and had almost disappeared by the end of the century [20], with the last recorded outbreaks (33 cases) reported as late as 1911 [17]. An improved drainage system created between 1863-1914 in the region of Lolland-Falster has been correlated with the decline in malaria incidence in the country [20]. Studies also suggest that improved livestock farming systems in the nineteenth century created a barrier between *Anopheles maculipennis* (the major carrier of vivax malaria in Denmark at that time) and humans, thereby contributing to a reduction in malaria cases in Denmark[19]. In addition, Setaria tundra, a mosquito-borne filarial nematode was identified in roe deer in Denmark [21], and Tick-borne encephalitis is sporadically reported in the country, with a higher incidence on the island of Bornholm [22]. Lyme borreliosis is the most common and dangerous tick-borne disease in humans in Denmark [23].

1. 3 Arthropod vectors in Denmark

A number of changes including global warming, global trade, and tourism are making northern Europe more vulnerable to the introduction of new arthropod vectors. Denmark is home to a number of malaria-carrying mosquitoes including *Anopheles maculipennis, A. plumbeus,* and *A. claviger* [19]. As recently as 2013, *Culex modestus,* a vector of West Nile virus was identified in Denmark; the most northern recording of this vector in Europe [24]. A number of *Aedes* mosquitoes are commonly found in Denmark including *Aedes vexans,* a vector of Rift Valley fever virus. *C. pipiens*, vector of West Nile virus is also common in Denmark (http://www.myggetal.dk/).

Culicoides biting midges are the most abundant hematophagous insects in Denmark. In addition, Obsoletus ensembles (*C. obsoletus*, *C. scoticus*, *C. montanus* Shakirzjanova, *C. chiopterus* (Meigen) and *C. dewulfi*), Pulicaris ensembles (*C. pulicaris* (Linnaeus) and *C. punctatus* (Meigen)) are the most abundant biting midges on ruminant farms [25]. *Culicoides* transmit a large number of pathogens to humans as well as domestic and wild animals. More than 50 viruses have been isolated from *Culicoide*s species globally [26]. Five different strains of BTV (1,4, 8, 9, 11) have been reported in northern Europe since 2006 [27,28] and Schmallenberg virus was detected in Culicoides vectors in Denmark in 2011 [8]. *Dermacentor reticulatus,* a new species of tick that can carry a number of zoonotic diseases (including *Rickettsia raosultii*) and animal pathogens (e.g. *Babesia canis*) was identified in Denmark in 2017 [29].

1. 4 Vector-borne disease transmission parameters

Epidemiological modeling plays a vital role in policy planning and predictions for controlling VBD[30]. Deciding what approach to take requires models able to generate accurate predictions, which in turn relies on an accurate knowledge of the natural lifecycle of vectors [30], including the daily survival probability, biting rate, extrinsic incubation period (EIP) of the virus, and the vector competence of the insect [30] .

1.4.1 Extrinsic incubation period (EIP):

The EIP is the time interval between ingestion of the infected blood meal and the time when the vectors are able to transmit the virus (or another pathogen) [31]. This is also known as “virus (or pathogen) development time”. Insect vectors are poikilothermic, and

the temperature of their environment is therefore a key factor affecting the rate at which an arbovirus is able to replicate to transmissible levels within the vector following ingestion [32]. For most vector-borne diseases, there is a threshold temperature below which the pathogen will not be able to develop within the vector. This threshold varies across different laboratory experiments for the same virus. For example, the threshold temperature for BTV varies from 10.4°C to 14.2°C [12,33]. The BTV-9 threshold of 13.3°C is widely used for Schmallenberg virus development as there are no experimental data available on this virus [34]. For Dirofilaria and another filarial nematodes, the threshold is considered to be around 14°C [35]. Above these threshold temperatures, the rate of virus development varies greatly. Some pathogens develop at a linear rate after the threshold temperature is exceeded, while some pathogens have a complicated nonlinear relationship at higher temperatures. Furthermore, some pathogens stop developing above certain temperatures (e.g. 34.4°C for malaria) [36]. A number of equations for EIP have been developed and are used for modeling different serotypes of BTV [12,32,33] (**Fig. 1**).

1.4.2 Daily survival rate:

The daily survival rate of adult females is one of the most important parameters determining mosquito- and *Culicoides*-borne disease transmission, as a slight increase may exponentially increase the total number of infectious bites from an insect [37]. The vectors must survive longer than the sum of the initial period (non-feeding) and the EIP to be able to infect a new host [37]. For BTV transmission, that means a lifespan of at least 15 days (2 days of non-feeding and 12-13 days for the virus development period) [37,38]. However, many scientists believe that the daily survival rate is independent of age and life span [39], while others believe it is age-dependent [40]. The life span of mosquitoes has been described as 24–67 days [41], and for biting midges between 10 to 30 days, but as long as 90 days under very cool weather conditions [26]. When modeling BTV, a number of equations have been used for the daily survival rate of *Culicoides spp* [33,42].

1.4.3 Blood meal digestion period/ gonotrophic cycle:

The blood meal digestion period is the time required to complete the cycle of blood feeding to egg-laying and back to blood feeding [12]. The digestion of blood meals is dependent on the temperature to which the insect is exposed. One of the key assumptions

is that the faster they digest the blood meal, the faster they will bite a host for another blood meal.

1.4.4 Vector competence:

Vector competence refers to the intrinsic ability of an arthropod to be infected, support replication and transmit a pathogen [12]. A number of physiologic, genetic, and environmental factors are involved in determining the competence of a vector. The transmission rate from host to vector and the transmission rate from vector to host play an important role in determining vector competence. Most BTV models use fixed values or a range of probabilities for these two transmission rates. BTV can transmit at a higher rate (0.7-1.0) from vector to host [43–46], while the transmission rate from host to vector has a much wider range: 0.002 to 0.64 [14,44–46]. Recently, a study conducted on South African biting midges provided a temperature-dependent equation to calculate the transmission from host to vector for *C. imicola* and *C. bolitinos* [45,47]. However, the two equations showed a considerable difference in the ability of biting midges to be infected with BTV.

1.4.5 The basic reproduction rate:

Popularly known as R_0, the basic reproduction rate is the number of secondary cases generated by an index case in a susceptible population. R_0 is considered a powerful tool when assessing the risk of disease invasion. A disease is able to cause an epidemic if the $R_0>1$, and R_0 can therefore provide a means to assess the level of risk posed by a disease [43]. The first R_0 model for VBD was developed by Ross in 1902, and the framework was fully developed by MacDonald in the 1950s [48,49]. In the Ross-Macdonald model, the R_0 is defined as:

$$\boldsymbol{R0 = \frac{ma^2bcp^n}{(-ln\,(p))r}}$$

where m is the vector-to-host ratio, a is the daily biting rate of the insects, b and c are the pathogen transmission efficiencies, p is the daily survival rate of mosquito, r is the recovery rate of the host, and n is the EIP in days of the pathogen in the insect.

1.4.6 Vectorial capacity:

The vectorial capacity of a VBD is defined as the number of new hosts infected from one infectious host per day [12,40]. Garret-Jones (1964) [50] emphasized vectorial capacity to avoid the difficulties associated with the duration of the infectious period of a host. The vectorial capacity can aid us in investigating when an outbreak could occur and how many animals could be infected. A simple version of the vectorial capacity, denoted as C and adapted from Garrett-Jones [50] is shown as:

$$C = m * a^2 * p^n * (\frac{1}{-lnp})$$

where m is the number of vectors per host, a is the biting rate, p is the daily survival probability of vectors, and n is the EIP of a pathogen in the vector. Vectorial capacity is likely to be useful when measuring the ability of the vector population to transmit a disease [12].

For many of the temperature-dependent parameters (e.g. EIP and survival rate), a number of different equations have been suggested for different strains of BTV [12,32,33,42] (**Fig. 1**). Using one equation could result in a rather different scenario than when another equation is used. Mathematical models using one equation for each parameter could either overestimate or underestimate the potential for BTV transmission. Such approaches do not take into account the uncertainties associated with the parameters and overall risk assessment.

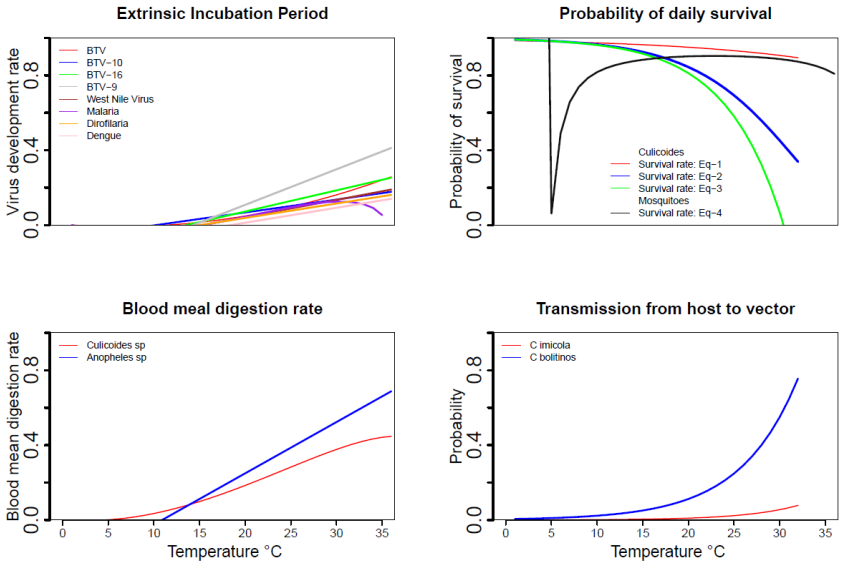

Fig 1: The relationship between temperature and different parameters often used in *Culicoides*-borne disease transmission models [12,33,42–47,51]

1. 5 Vector-borne disease models:

In 1902, Sir Ronald Ross was awarded the Nobel Prize in Medicine for his work on malaria. He published a series of works on the mathematical explanation of malaria transmission in 1908 and 1911 [52,53]. After the Second World War, Macdonald picked up Ross's work and developed a full mathematical model for malaria, known as the Ross-Macdonald Model [48]. This work is still considered the foundation for a number of models used to study vector-borne diseases. Today, the models have been developed for different VBD [43,54–56], but the core biological assumptions remain true to the original Ross-Macdonald model. The Ross-Macdonald formula uses a fixed rate for each parameter (e.g. EIP) to estimate the basic reproduction rate. For example, the duration of the EIP is a fixed number of days, and mosquitoes/biting midges can only become infectious after that period.

1.5.1 Biological process-based cohort model:

A biological process model follows the biological phenomenon of vectors and hosts independently, and identifies the number of infected hosts originating from an infectious host. The model follows this cohort until all vectors are dead. The model here runs for one cohort at a time, starting with the cohort that bites on the first day of the selected time period, and runs successively through the remaining days of the time period. During each run, the model calculates different events such as the daily survival rate, EIP, and biting rate in each cohort. The model identifies the date when mosquitoes in each cohort become infectious (i.e. when the EIP is completed), based mostly on rate summation. The model calculates and identifies the dates when the vectors complete each gonotrophic cycle for each daily cohort. It is assumed that the mosquitoes will take a new blood meal at the end of each gonotrophic cycle. The model identifies the date of the infectious bite in each cohort by comparing the dates when vectors bite after the EIP is complete. This date is then merged with the information on survival rates to calculate how many vectors of the original cohort have survived until that day. The number of surviving vectors represents the number of new infectious bites by the vectors in the specific cohort. The model gives three estimates: 1) the date the cohort became infected, 2) the date that bites from the cohort result in infection, 3) the number of infectious bites from the cohort each day. The model can therefore estimate how many new infectious bites can be generated from a daily cohort of biting midges. Finally, to estimate the basic reproduction rate per animal, the model can sum up the number of infectious bites generated during the infectious period of the host animals.

This deterministic approach follows every stage of the animal/vector with real-time data (temperature, vector density etc.). In such a process model, parameters are correlated with each other. For example, if a cohort of mosquito is modeled with a higher temperature each hour, the result will be a shorter EIP and higher biting rate, but with a reduced daily survival rate. However, process models are very detailed and it becomes difficult to run simulations in larger datasets.

A biological process-based cohort model was used in this PhD, with uncertainty included in the variables (e.g. different microclimatic and meteorological temperature, variation in vector abundance, more than one equation for the VBD transmission parameters including EIP, survival rate, and probability of transmission from host to vector; Manuscript III).

However, it should be noted that other modeling approaches are also popular in VBD, including the compartmental model.

1. 6 Temperature, meteorological temperature and microclimatic temperature

Temperature has a substantial effect on vector-borne disease transmission [57,58]. Many of the lifecycle events of insects (including the daily survival rate, biting rate, pathogen development rate, and vector competence) are all a function of temperature [12,31]. Mathematical models used to quantify disease transmission dynamics are mostly driven by temperatures recorded by meteorological institutes. Regular meteorological observations began in Europe in the second half of the nineteenth century and modeler started using these in disease transmission models from the mid-twentieth century – particularly for diseases sensitive to temperature, such as VBD [48,57]. The Ross-Macdonald model for malaria was one of the first models to be described for VBD transmission [48]. Detinova et al. (1962) established a relationship between temperature and malaria parasite development [57]. The relationship between temperature and lifecycle event of insects (including survival rate, biting rate, and transmission rate from host to vector) was later established, confirmed and elaborated [49,59–62].

1.6.1 Meteorological Temperature:

After the World Meteorological Organization (WMO) was established on March 23, 1950, a standard was set among its member states to record the temperature using weather stations. In accordance with these standards, weather stations were set up at very specific heights all over the world (generally 2 m above the ground), using a white box placed in such a way as to protect the thermometer sensor from direct exposure to sunlight. The method used for measuring the temperature ensures that it is representative of a large area (i.e. 100 to 1000 km^2) [63].

Using meteorological temperatures in a disease transmission model may introduce potential bias. Insects are poikilothermic and they experience temperatures at a very local level, down to a scale of meters or even centimetres [64]. Models using meteorological temperatures ignore the temperature to which the insects are exposed in the microhabitats [65,66]. Weather stations

may be located as far as 50–100 km from the insect resting sites, and the meteorological temperature recorded will represent only one of many potential microclimates (the standard microclimate) of the area. While meteorological temperature is recorded at a specific height, the insects might rest at different heights and in different habitats [65,67,68]. The lack of data from microclimatic environments hinders the use of microclimatic temperature in disease modeling.

In this PhD, I recorded the microclimatic temperature from two or three different heights at six potential insect habitats (dry meadow, wet meadow, hedges, forest, cattle grazing field, and horse grazing field) in Denmark. I compared this to the meteorological temperature recorded from the nearest Danish Meteorological Institute (DMI) weather station, and compared the impact of using both types temperature on the EIP of six pathogens (Manuscript I).

1.6.2 Microclimatic temperature:

Microclimatic temperature was first described in the early part of the last century by German plant ecologist Gregor Kraus, who used the German word "Kleinstklima" shortened to "Kleinklima" meaning "microclimate", hence he is known as the father of microclimatology. He described microclimatic temperature as being different from meteorological temperature, and took initiatives to measure it near to the ground and at various other heights[69]. German meteorologist Rudolf Geiger (1950) defined microclimatic temperature in his book *The Climate Near the Ground* as the "climate in the least space" [69] and, "the set of atmospheric conditions that occur in a local area as a result of environmental heterogeneity near the earth's surface" [69,70]. Geiger described the mechanisms of heat exchange at the ground, heat conduction, the warming and cooling process, and the relationship between ground surface temperature and factors affecting this temperature, such as humidity, wind, and solar radiation.

Microclimatic temperature and its impact on ecology and disease transmission have been studied in agriculture, forestry and ocean science [69–74]. In vector-borne disease modeling, the use of microclimatic temperature is still in an early phase. At the beginning of this century, a number of studies were conducted on microclimatic temperature and its role on vector-borne disease transmission, in particular focusing on Malaria [36,75], leishmaniosis [76], and dengue [66].

Most of these studies compared the temperature of one particular type of microclimatic habitat with local weather station temperatures. Studies in Chennai showed that daily mean temperatures in urban areas were generally warmer than those recorded at the local weather station [36]. In a study from Argentina, microclimatic conditions in domestic and peri-domestic habitats were generally 5.0°C to 5.6°C higher than the ambient temperature [76].

While modeled meteorological temperatures are available on a very local scale (1 km^2) in Denmark, microclimatic temperatures are not. The microclimatic temperatures of habitats surrounding livestock farms are important in assessing the impact on vectors and consequently vector-borne diseases infecting livestock (e.g. BTV and Schmallenberg virus). The microclimatic temperature of different potential insect habitats should therefore be studied at different heights. A small number of studies have used meteorological parameters to predict the temperature of microclimatic habitats including forest microclimates [70] and substrates (soil, rock, and sand) [71]. Many of these models identified some climatic variables (including temperature, solar radiation, humidity, rainfall and wind speed) as being important in predicting microclimatic temperatures. However, there are no models available to predict the microclimatic temperature of potential mosquito and *Culicoides* habitats.

In this PhD, I modeled the microclimatic temperature of six different potential insect habitats in Denmark (dry meadow, hedges, wet meadow, forest, cattle and horse grazing filed). This was based on the available meteorological parameters from the Danish Meteorological Institute, which included hourly temperature, solar radiation, humidity, wind speed, and rainfall.

1. 7 Insect resting sites

Insects spend almost 90% of their lifetime resting while digesting blood meals and developing eggs [77]. Little is known about the resting sites of biting midges and mosquitoes in Denmark, but different species of biting midges may prefer different types and heights of vegetation [67]. In other countries, biting midges were found in different quantities in wetlands, peat bogs, riverine areas and livestock farms, but higher numbers were recorded in wetland areas [65]. The insects seek favorable microhabitats, and their choice is determined by the temperature and humidity of the location [78]. Carpenter (1951) found adult biting midges distributed equally at 2.2 m, 7.0 m and 10.7 m above ground [79]. We know a very little about

the resting behavior of insects at low Scandinavian temperatures [77], but they are able to move between different resting sites in order to optimize the conditions. Kirkeby et al. [80] recaptured marked biting midges at a distance of up to 1.75 km from their release point in Denmark. In this PhD, I hypothesized that biting midges choose resting sites either randomly or based on the temperature of the microclimatic habitats (warmest or coolest). To adopt a conservative approach and for comparison with other published studies, I also assumed that insects will rest in a habitat where temperature is very much comparable to that of the Danish Meteorological Institute recorded/modeled temperature (Manuscript II).

1. 8 Setaria tundra outbreaks in Finnish reindeer

S. tundra is a mosquito-borne filarial disease of reindeer (*Rangifer tarandus tarandus*) that causes peritonitis, peri-hepatitis and poor body condition. *Aedes spp.* and *Anopheles spp.* mosquitoes are the main vectors responsible for the transmission of *S. tundra* in Finnish reindeer-herding areas [11].

In Lapland in northern Finland, reindeer are reared as semi-domesticated animals. The area has a short summer season of 2-3 months, which is thought to be unsuitable for large VBD outbreaks, yet reindeer are frequently infected with *S. tundra*. At least three large outbreaks of *S. tundra* have been documented in Finnish reindeer since 1973 [9]. Recent studies have shown a correlation between higher mean temperatures of two successive summer seasons and *S. tundra* outbreaks in Finland [10]. While many other studies on vector-borne diseases have suggested a similar correlation between increasing temperatures (global warming) and disease outbreaks [81–84], the exact mechanism of how increasing temperatures can cause such outbreaks is not well described. However, this could be attributed to: i) an increase in the duration of the annual transmission periods allowing more pathogen generations, ii) faster pathogen development time in vectors, iii) increased vector abundance leading to increased mosquito biting rates. In this PhD, I investigated how increasing temperatures may have affected the annual accumulated transmission of the infective stage of *S. tundra* larvae to reindeer hosts (Manuscript IV).

Reindeer are reared as a semi-domesticated animal in 54 different cooperatives in Finland. Most of the animals are slaughtered from September to December, but a small number may be slaughtered as late as May the following year. Of the slaughtered reindeer, 80% are born the same year, and 20% are old reindeer [85]. Most of the reindeer (~70-80%) are slaughtered

in officially approved abattoirs, while the remaining animals are slaughtered privately at home by the owners [85,86]. Meat inspection has been performed regularly since the 1980s [85,86], but data on liver condemnation due to *S. tundra* have only been recorded since 2004. A correlation between higher mean temperatures of two successive summer seasons and *S. tundra* outbreaks in Finland has been identified [10]. This thesis explores how increasing temperatures may have affected the annual accumulated transmission of the infective stage of *S. tundra* larvae to reindeer hosts.

There is a difference between modeling vector-borne viral and filarioid parasite diseases. Mosquitoes ingest a specific number of microfilaria while taking a blood meal from infectious reindeer, and these microfilaria do not multiply in the vector but will turn into an infective stage called L3 after the EIP is complete. The mosquito will be able to transmit this fixed number (or a fraction of this number) to a new reindeer while taking another blood meal. In this case, there would be no worms left to transmit during a subsequent bite. In contrast, for vector-borne viral disease (e.g. in the basic reproduction rate model for BTV and Schmallenberg virus [43,87]), all bites are considered to be infectious after the EIP is complete because the virus replicates in the vector, although the virus load is not quantified.

1. 9 Climate change and vector-borne diseases:

The Intergovernmental Panel on Climate Change (IPCC) projected the mean global surface air temperature will increase to 5.8°C by 2100, an increase of 1.4°C from 1990 [88]. A recent study showed that there is only a 5% chance that global temperatures will increase by less than 2°C by the end of the century [89]. In Europe, 63% of infectious diseases are climate-sensitive, and 82% of these are sensitive to temperature alone. Furthermore, vector-borne diseases have been identified as the most temperature-sensitive diseases [81]. The WHO estimates that VBD is responsible for around 16% of all illness and disability suffered worldwide, with more than half of the world's population currently at risk of VBD infection [90,91].

Current evidence suggests that global warming will affect the transmission of vector-borne diseases in terms of a broader geographical expansion of existing disease and a higher number of outbreaks in endemic regions [83,92], as increased temperature could result in an increase in the reproduction and biting rates of the insect, while shortening the pathogen incubation period [93]. It has been suggested that the emergence of BTV in central, western

and northern Europe at beginning of this century was driven by recent changes in the European climate that allowed increased virus persistence during winter [83]. Furthermore, the northward expansion of *C. imicola*, and transmission by indigenous European *Culicoides* species including *C. obsoletus and C. pulicaris* allowed for the transmission of BTV over larger geographical regions [83].

However, the ultimate impact of VBD will depend on how the insects behave in increased temperatures. If they choose to rest in colder microhabitats, the real impact of global warming might not be as serious as currently predicted. However, if they choose resting sites randomly, or choose warmer sites, the impact of global warming may be much more severe than anticipated.

In this PhD, I hypothesize that insects choose resting habitats randomly or based on microclimatic temperature (warmest or coolest) every hour. I then evaluate the impact of the temperature at different resting sites in Denmark on the EIP of Schmallenberg virus on a temporal and spatial scale (Manuscript II).

1. 10 Aim, goals, and objectives of the thesis

The overall aim of this thesis was to improve our understanding of the transmission potential of vector-borne diseases in northern Europe.

In order to meet the overall aim, four goals were defined for the work presented in this thesis:

- Goal 1: Quantify the difference between meteorological and actual microclimatic temperatures of potential insect habitats (mosquitoes and biting midges) and quantify the impact on the extrinsic incubation period (EIP) of vector-borne diseases:

    - Objective 1.1: Compare the microclimatic temperatures of insect habitats (dry meadow, hedges, wet meadow, forest, cattle grazing field, horse grazing field) and their nearby meteorological institute weather station data.
    - Objective 1.2: Compare the seasonal impact of microclimatic and meteorological temperatures on the development time of a number of vector-borne diseases (bluetongue, Schmallenberg, West Nile and dengue virus, vivax malaria, Dirofilaria parasites) and on the blood meal digestion period of biting midges and mosquitoes.
    - Objective 1.3: Develop a model that can predict the microclimatic temperature of habitats based on readily available parameters from the local meteorological institute.

- Goal 2: Estimate the microclimatic temperature of habitats surrounding all Danish cattle farms, compare the impact of that temperature on Schmallenberg virus development time in relation to that of meteorological temperature, and identify the spatial and temporal patterns of Schmallenberg virus development time on the Danish cattle farms:

    - Objective 2.1: Estimate the microclimatic temperature of habitats within a 500m radius of all cattle farms in Denmark (n=22,004) for the period 2000-2016.
    - Objective 2.2: Compare the Schmallenberg virus development time using both meteorological and microclimatic temperatures.

- Objective 2.3: Describe the spatiotemporal pattern of Schmallenberg virus development time around Danish cattle farms.
- Objective 2.4: Compare the role of habitat (insect resting sites) on Schmallenberg virus development time in Denmark.

- Goal 3: Assess the risk of BTV outbreaks in Denmark:

  - Objective 3.1: Describe the *Culicoides* distribution data in Denmark.
  - Objective 3.2: Estimate the number of daily infectious bites from *Culicoides* infected with BTV in Denmark using different microclimatic temperatures.
  - Objective 3.3: Assess the impact (sensitivity and uncertainty) of different parameters on BTV transmission (EIP, survival rate, biting rate, and probability of transmission from host to vector).

- Goal 4: Study the role of temperature on *Setaria tundra* outbreaks in Lapland, Finland:

  - Objective 4.1: Describe the spatial and temporal pattern of *S. tundra* outbreaks in three different areas from south to north across Finnish Lapland.
  - Objective 4.2: Construct a temperature-driven mechanistic transmission model to quantify the potential role of temperature on the occurrence of *S. tundra* outbreaks and on the intensity of the worm burden in reindeer in general.
  - Objective 4.3: Identify a trend in the effective transmission season duration and potential average worm burden in Finnish reindeer.
  - Objective 4.4: Test whether the estimated microclimatic temperature can explain disease transmission in the absence of actual recorded microclimatic temperatures in settings other than Denmark.

In order to fulfill these aims and objectives, four studies were conducted that led to four scientific manuscripts entitled:

1. Manuscript I: Microclimatic temperatures increase the potential for vector-borne disease transmission in the Scandinavian climate. Published, Scientific Reports, 7, 8175.

We recorded microclimatic temperatures from two or three different heights in six different microclimatic habitats and compared these with recorded temperatures from the nearest meteorological weather station. We then compared the impact of the hourly temperature on the extrinsic incubation period (EIP) of six vector-borne pathogens. Finally, we developed a regression model to predict the microclimatic temperatures of different habitats based on standard meteorological parameters (hourly temperature, solar radiation, humidity, wind speed, rainfall) readily available from the local meteorological institution.

2. Manuscript II: Microclimatic temperatures at Danish cattle farms, 2000–2016: quantifying the temporal and spatial variation in the transmission potential of Schmallenberg virus. Published in Parasite & Vectors, 11, 128.

   We estimated the hourly microclimatic temperatures at potential vector-resting sites within a 500 m radius of all Danish cattle farms from 2000 to 2016. We then modeled the daily EIP of Schmallenberg virus at each farm, assuming that vectors choose resting sites either randomly or based on temperatures (warmest or coolest available) every hour. Finally, we assessed the impact of insect resting sites on Schmallenberg virus development time in Denmark.

3. Manuscript III: Quantifying the potential for bluetongue virus transmission in Danish cattle farms. In preparation for the journal “Scientific Reports”.

   We used 17 years of temperature records from four microclimates for all Danish cattle farms to identify four sets of temperature for each hour of the warm season (April 1^{st} to October 31^{st}). Thus from this large sets of temperature for each hour we estimated the mean of fourth quantiles (>75^{th} percentiles), third quantiles (>50^{th} percentile but ≤75^{th} percentile), Second quantiles (>25^{th} percentile but ≤50^{th} percentile) and first quantile (≤25^{th} percentile) temperature. In addition to quantify the extreme condition we identified the maximum and minimum temperature for each hour. We used similar weekly *Culicoides* data (maximum, minimum, mean of 4^{th}, 3^{rd}, 2^{nd} and 1^{st} quantiles) from 1,453 trap collections between 2007 and 2016 at different locations in Denmark to estimate the number of daily infectious bites from

Culicoides infected with BTV. Finally, we performed a sensitivity analysis for each parameter/equation that plays a role in BTV transmission.

4. Manuscript IV: The annual, temporal and spatial pattern of *Setaria tundra* outbreaks in Finnish reindeer: a mechanistic transmission model approach. Submitted to "Parasites & Vectors".

   We collected daily maximum and minimum meteorological temperature data from the Finnish Meteorological Institute for the period 1979-2016 and converted these into hourly temperatures. We estimated the hourly microclimatic temperature following the suggestion from our first study and used both temperatures in a temperature-driven deterministic transmission model to estimate the average potential worm burden for reindeer each year. We then compared the model output from both types of temperatures to the liver condemnation rate due to *S. tundra* in reindeer in three locations of Lapland. Finally, we performed a time series analysis to test whether the effective transmission season duration in Finland followed a significant trend.

Chapter 2: Materials and methods

2. Materials and methods

The material and methods specific to each of the different studies are described in the "Method" section of each manuscript Chapter 3 (Results). This chapter will complement the background work done during the microclimatic temperature data collection, and the procedure for selection of meteorological variables needed from DMI in order to use them in modelling microclimatic temperature. There will be a short description of the biological process model, the estimation of daily infectious bites (IB) of BTV and slaughterhouse data collection from Finnish reindeer cooperatives.

2.1 Microclimatic data collection

We recorded microclimatic temperature at six habitats at Strødam and Faxe in Denmark during May1st-October 31st, 2015. At Strødam (N55.96007°, E012.27465°), we worked in a natural reserve area where a number of different potential insect habitats were available. We selected four different habitats including dry meadow, hedges, wet meadow, and forest for recording of microclimatic temperatures using data loggers named "Temperature logger (21G) [94] ([TM])". In each habitat, we collected temperature from three different locations (replicates) (**Fig 1**). At each height, we placed two data loggers in one small transparent plastic bag after removing the air manually. In Faxe, we recorded the temperature from a cattle grazing field close to a wheat field (N 55.20258, E012.11440). We also recorded temperature from a horse field close to a horse stable (N 55.22383°, E012.04494°). All together we deployed a total of 66 data loggers to record microclimatic temperature in the different habitats **(Fig 2A and 2B)** each hour and we transferred the data to a portable data recorder "TempTec-R" [95] at 15-30 day intervals.

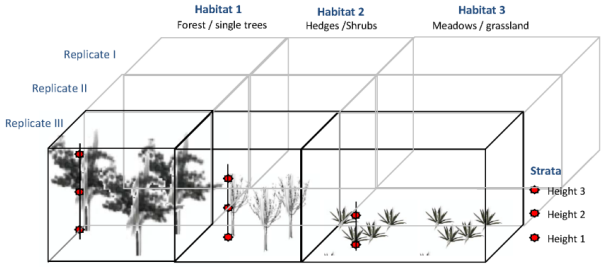

Fig 1: Sampling scheme for forest, hedges and wet meadow sampled in different strata (two or three heights). Each habitat type was sampled in three replicates with two loggers being placed at each height.

Fig 2A: The dry meadow habitat with the iron poll containing temperature data loggers, Strødam, Denmark

Fig 2B: The hedges habitat with the plastic poll containing temperature data loggers, Strødam, Denmark

2.2 Weather station data collection

In Strødam (N55.96007°, E012.27465°), we set up a portable weather station (Davis Vantage Pro 2) **(Fig 3B)** to record meteorological data (hourly temperature, solar radiation, wind speed, humidity, and precipitations) in order to identify the important variables for modelling microclimatic temperature that should be requested from DMI. The weather station recorded data at each hour and we transferred the data to a laptop at 15-30 day intervals **(Fig 3B)**.

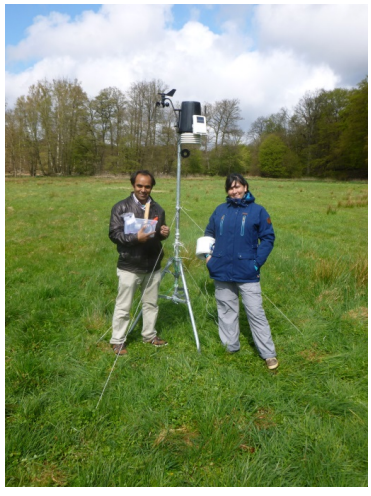

Fig 3A: The author with a PhD colleague setting up a weather station at Strødam, Denmark

Fig 3B: Downloading the weather station data from Strødam, Denmark

2.3 Selection of meteorological variables to be requested to DMI

Initially we performed a simple linear regression model to identify the important meteorological variables necessary to model microclimatic temperature using the weather station data. We identified that hourly temperature, solar radiation; humidity, wind speed and precipitation were important variables and could explain 64-72% of the variation in temperature observed in different microclimatic habitats at Strødam. We found it difficult to handle the variables "day" and "month" in the model. Although each "day" of a month plays a role in microclimatic habitats, it was not practical to use each "day" as a categorical variable. If we did not include "day" in the model but included "month" as a categorical variable, the model considered each day of a month to have a similar impact on estimating hourly temperature. For example, the temperature on 31st of May is more likely to be influenced by both May and June than just May [38]. Likewise, the length of grass in a habitat on 30th of June will be more similar to the grass length on 1st of July rather than 1st of June. If we ignore this relationship, the model will not be able to capture the continuous changes occurring in the microclimatic habitats. Therefore we used a floating weight for each day, giving the 15th day of each month (taken to be the mid-point) a weight of 1, with other days influenced by neighboring months, as expressed by the formula [38]:

$$\text{Month}(x)=15-(\text{absolute}(\text{Date})/\text{No. of days of month})$$

This is a 30 or 31-day window and the sum of the weights of each day is always 1. To avoid the negative values originating beyond these 30 or 31 days, all negative weights were set to 0. Thus the variable gave a relative importance of the temperature of the neighbouring days instead of the month (**Fig 4**).

Another variable we found important was the temperature of the previous hour (lag1 temperature). Microclimatic habitats take time to change and if, for example, solar radiation heats up the vegetation and soil in the habitat, it will take time to cool down, while the standard meteorological temperature 2 m above ground can change much more rapidly. By adding these two variables in the linear regression model, the model could explain 87-96% of the variation in microclimatic habitats.

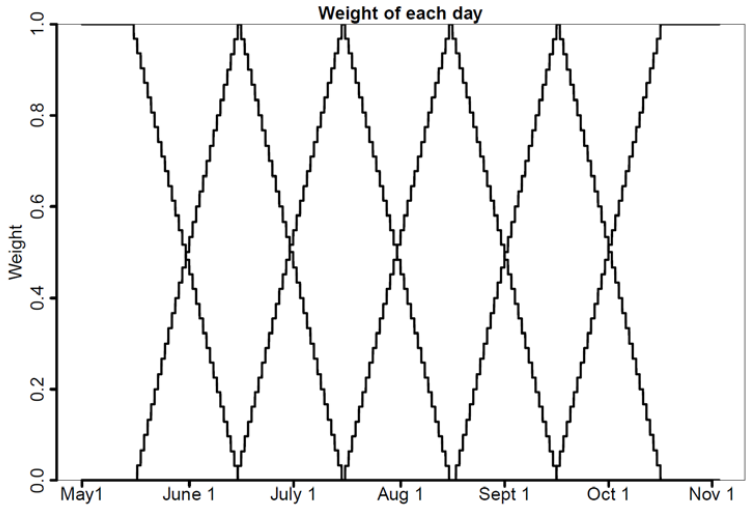

Figure 4: The weighting of each day of the month used in the linear regression model for converting DMI temperatures to microclimatic temperatures. The first 15 days of May and the last 15 days of October had no effects from neighboring months (as no data were available from April and November in our model) and therefore had a weight of 1. Each day of the study period had a cumulative weight of 1.

After selecting the important meteorological variables, we worked in collaboration with DMI and obtained meteorological data initially for two locations (Sjælsmark and Faxe Ladeplads) for the period of May to October of the year 2015. The DMI weather station at Sjælsmark (N 55.94407, E 12.27065), was 1.8 km away from the Strødam data collection point. We compared the meteorological data we collected by our weather station (denoted as "DTU") and the meteorological data obtained from DMI. The correlation between the parameters recorded for the two weather stations (DMI and DTU) was 0.89 for temperature, 0.79 for solar radiation, 0.83 for humidity and 0.67 for wind speed and 0.20 for precipitation (**Fig 5**). The data collected from DMI was used in the subsequent analysis in manuscript I. After modelling the microclimatic temperature for two locations of the year 2015, we further collected meteorological parameters from DMI for 17 years across Denmark (320 grid points). These data were used in manuscript II and III.

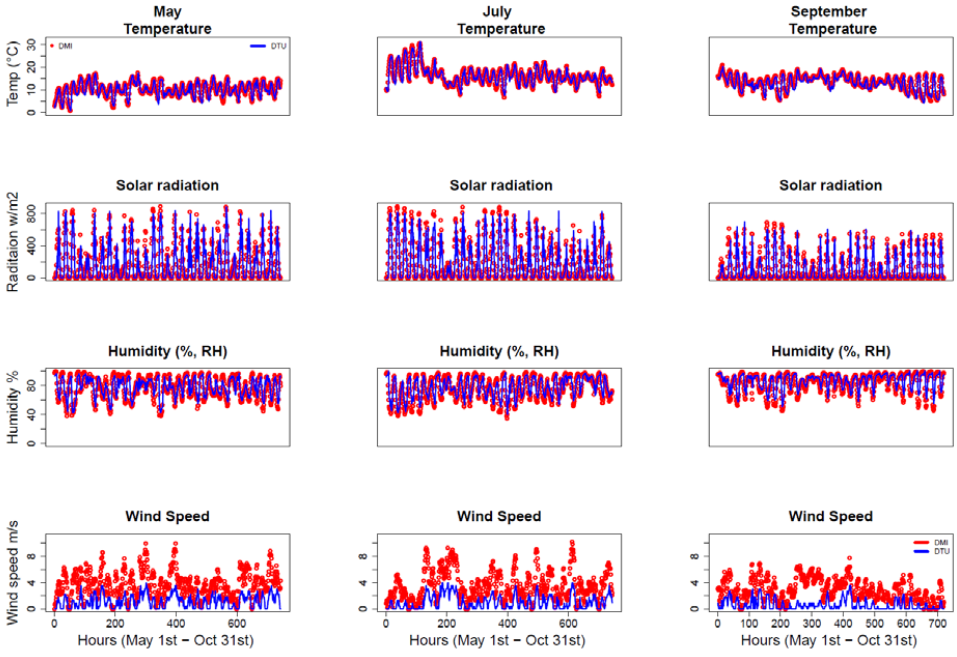

Figure 5: The comparison of hourly meteorological parameters recorded by the Danish Meteorological Institute (DMI) at Sjælsmark, Denmark and our weather station (DTU) set up at Strødam, Denmark for the period of May 1st to October 31st, 2015. The two locations were 1.8 km apart.

2.4 Biological process model:

We used a biological process model in this PhD research for modelling BTV and *S. tundra*. A biological process model is a deterministic approach that follows the biological events of the insect and host independently. The model then estimates the number of secondary infected hosts (or IB) from one infectious individual via the insect vectors. The model follows a cohort of insects each day of a transmission season until they die. During the progression of an insects life, the model identifies how long the insect will survive, when the EIP will be completed, when the insect will bite, and how many times the insect will bite during its lifespan. The model further follows the rate of pathogen transmission from host to vector and from vector to host. The daily survival rate of the insect, the biting rate of the

insect, and the EIP and rate of transmission of pathogen from host to vector are all a function of temperature [31–33,45,47]. The model runs for one cohort at a time, starting with the cohort that bites on the first day of the selected time period, and runs successively through the remaining days of the time period. The model can therefore estimate how many new IB can be generated from a daily cohort of biting midges. The model can further be used to estimate the R_0. In order to estimate the R_0 the duration of viremia of pathogens are considered. For example the mean duration of viremia of BTV in naturally infected cattle is 20.6 days [96] and in order to estimate the R_0 all the IB generated among these 20.6 days are to be summed up.

There are differences between modelling vector borne viral diseases and vector-borne filarial diseases. In filarial diseases, mosquitoes can transmit only a fixed number of larvae (worms) that they have ingested (in the microfilaria stage) while taking a blood meal from an infectious host, because microfilaria do not replicate within the mosquito [35,97,98]. In case of vector borne viral diseases (e.g BTV, Schmallenberg virus) all the bites after completion of the EIP are considered infectious as the virus replicates within the insects (although the viral load is not quantified) [34,43,51,99]. We have considered this difference while estimating the amount of larvae being transmitted from an infectious reindeer (Manuscript –IV). When modelling BTV, we quantified transmission as the number of IB where all bites have the same risk. When modelling S. *tundra*, we quantified transmission as the number of infectious units (number of L3) transferred since each bite varies in the number of infectious units.

2.5 Estimation of daily IB for BTV:

We estimated the number of daily IB of BTV using a series of temperatures, *Culicoides* abundance data along with differing equations for EIP, daily insect survival rate, biting rate, and probability of transmission from host to vector.

We estimated a distribution of IB for each day rather than a point estimate. Instead of performing a simulation with each parameter, we considered all the available equations of each parameter so that we could capture all the possible variation.

2.5.1 Temperature:

We used microclimatic temperature to estimate the daily number of IB as these are the actual temperatures to which the insects are exposed[38,65]. We obtained hourly meteorological parameters (temperature, solar radiation, humidity, and wind speed) for the period 2000-2016 from DMI at 320 grid points across Denmark. The nearest grid point to each of the 22,004 cattle farms was considered to be the temperature at that farm. The type of land cover within a 500 m radius of each cattle farm was quantified using CORINE Land Cover [100]. The land cover was reclassified as dry meadow (83%), hedges (6%), wet meadow (3%), and forest (3%) [101]. Using our published microclimatic model [38,101], these meteorological parameters were converted into hourly temperatures for the period of 1st of April to 31st of December for each of the four different microclimatic habitats. This resulted in four different hourly temperatures for 22,004 cattle farms over the 17 years. From these large datasets (17 years × 22,004 cattle farms) we selected six series of temperature data for each of the four microhabitats including:

i) Maximum temperature: For each hour, the maximum temperature recorded at any cattle farm of the country during 2000-2016
ii) Minimum temperature: For each hour, the minimum temperature recorded at any cattle farm of the country during 2000-2016
iii) First quantile mean temperature: for each hour, the temperature below the 25th percentiles among all the 22,004 cattle farms during 2000-2016
iv) Second quantile mean temperature: for each hour, the temperature equal to and above the 25th percentile and below the 50th percentile among all the 22004 cattle farms during 2000-2016
v) Third quantile mean temperature: for each hour, the temperature equal to and above the 50th percentile and below the 75th percentile among all the 22,004 cattle farms during 2000-2016
vi) Fourth quantile mean temperature: for each hour, the temperature above the 75th percentile among all the 22,004 cattle farms during 2000-2016

The maximum and minimum temperature data were used only for estimation of the best and worst case scenario and were not considered in the general estimation of the daily number of IB.

2.5.2 *Culicoides abundance data*:

We obtained a total of 1,463 trap collections over 1- 3 nights from 351 cattle farms across Denmark between 2007 and 2016 from different studies run by the Technical University of Denmark. Where collections were made on more than one night, the daily mean number was used for each day, with the assumption that the trap would collect midges equally each day. Details of the counting and species-identification (Obsoletus ensemble and Pulicaris ensemble) methods are described in Kirkeby (2012) [102] and Lassen et al. (2012) [77]. All data were available for this study. We identified the week number for each collection date, considering 1st of January as the first day of the year. We calculated six series of weekly *Culicoides* data similar to the above described temperature data.

We calculated the minimum, maximum, mean of first quantile (> minimum and <25th percentile), second quantile (≥25th percentile <50th percentile), third quantile (≥50th percentile <75th percentile) and fourth quantile (≥75th percentile) number of Obsoletus and Pulicaris ensembles separately from the weekly distribution of trap data. We then generated a daily abundance for each of the six estimates for each species ensemble by assuming the abundance would be the same on each day of the week. Finally, we smoothed each of the 12 daily *Culicoides* abundance (six Obsoletus and six Pulicaris) data by a 14-day running average and used these 12 estimates as averages of the daily vector abundance on cattle farms in Denmark over an average season.

The maximum and minimum *Culicoides* data were used only for estimation of the best and worst case scenario and were not considered in the general estimation of the daily number of IB.

2.5.3 *Daily survival rate:* We used three equations for daily insect survival rate and ran the model independently for each equation [33,42]

2.5.4 *EIP:* We used four equations for EIP and ran the model independently for each equation [12,28,32,33]

2.5.5 Insect biting rate: We used one equation for the completion of the gonotrophic cycle and ran the model independently for each equation. After completion of each gonotrophic cycle, the biting midges will bite a new host on the next day[12]

2.5.6 Host to vector transmission rate: We used two equations for transmission of BTV from host to vector for the Obsoletus

ensemble. In addition, we also used a fixed rate of transmission from host to vector for both Obsoletus and Pulicaris ensembles (0.071 and 0.0401 respectively) [45,47].

2.5.7 Vector to host transmission rate: *The rate of transmission from vector to host was set to be 0.80 for both the Obsoletus and Pulicaris ensembles* [103]

We ran the model using all combinations (4 temperatures × 4 habitats × 4 vector abundance values × 4 EIPs × 3 survival rates × 3 host-to-vector transmission rates), resulting in a total of 2304 different numbers of IB for each day of the transmission seasons for the *Obsoletus* ensemble. For the Pulicaris ensemble, we used only one rate of transmission from host to vector, and thus our model estimated 768 different numbers of IB for each day. Thus for each day we had a total of 3,072 numbers of IB estimated with different combinations.

To identify the worst- and best-case scenarios, we also ran the model with the maximum and minimum temperatures along with the maximum and minimum abundance of biting midges, respectively. This gave another 144 estimates for each of the best and worst scenario (4 EIP × 3 survival rate × 3 rate of transmission from host to vector × 4 habitats) for the Obsoletus ensemble and 48 estimates for each of the best and worst scenario (4 EIP × 3 survival rate × 4 habitats) for the Pulicaris ensemble. We identified the worst case as the daily maximum value of IB for each day from the 144 different estimates. Similarly, we identified the best case as the daily minimum value of IB for each day from the 144 different estimates.

Finally, we made an adjustment regarding land cover type, since it is more likely that the insect will rest in the habitats that are more available to them. Since 83% of the land cover surrounding the Danish cattle farms were dry meadow, and hedges were 6% compared to 3% for both forest and wet meadow, we adjusted our model output by replicating the daily IB estimates 7 times by those estimated from dry meadow temperature, 2 times with those estimated from hedges and used the complete dataset including the IB estimated from wet meadow and forest temperature in a summary analysis.

2.6 Data collection from Finland

Reindeer (*Rangifer tarandus tarandus*) husbandry is an integral part of the Arctic culture in Finnish Lapland [104]. *S. tundra* is a mosquito-borne filarioid parasite

disease of reindeer. The disease is associated with peritonitis, perihepatitis and poor body condition [9–11], and therefore has a negative impact on the welfare of reindeer [97]. In order to understand the husbandry practice and to collect data on slaughterhouse inspection of reindeer, the author stayed two weeks in Kuusamo, one of the southern regions of the Finnish reindeer husbandry area. During his stay, he visited an autumn round-up, when animals are brought together in a fenced area to select the animals for slaughtering and to deworm the animals left for breeding (**Fig 6A**). The author also observed the slaughterhouse inspection of reindeer (**Fig 6B**). The slaughterhouse data were collected under the project "Reindeer health in the changing environment 2015-2018", funded by the Finnish Ministry of Agriculture and Forestry (MAKERA). The degree of peritonitis/perihepatitis due to *S. tundra* is measured on a scale of 0 to 3: 0 no visible changes; 1 moderate change with only slight local fibrin formation on the surface of the liver or peritoneum, resulting in only minor parts of the organs being condemned; 2 severe changes, with fibrin formation on the liver and peritoneum resulting in the whole liver and peritoneum being condemned , 3 very severe changes in which the whole liver or peritoneum is covered with a thinck fibrinous membrane giving an impression of a purulent process, the quantity of the abdominal fluid is increased and there are adhesions between the mesentery and other visceral organ [85]. In our analysis we considered all the animals whose organ(s) fall under the scale 2-3. The meteorological data for the period of 1979-2015 were ordered from the Finnish Meteorological Institute through email communication.

Fig 6A: The author in front of a reindeer herd gathered for autumn round up, Kuusamo, Finland

Fig 6B: Inspection of slaughtered reindeer organs for *S. tundra* , Kuusamo, Finland

Chapter 3: Results

Manuscript I

Microclimatic temperatures increase the potential for vector-borne disease transmission in the Scandinavian climate

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Microclimatic temperatures increase the potential for vector-borne disease transmission in the Scandinavian climate

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We quantified the difference between the meteorological temperature recorded by the Danish Meteorological Institute (DMI) weather stations and the actual microclimatic temperatures at two or three different heights at six potential insect habitats. We then compared the impact of the hourly temperature on the extrinsic incubation period (EIP) of six pathogens. Finally, we developed a regression model, enabling us to predict the microclimatic temperatures of different habitats based on five standard meteorological parameters readily available from any meteorological institution. Microclimatic habitats were on average 3.5–5 °C warmer than the DMI recorded temperatures during midday and 1–3 °C cooler at midnight. The estimated EIP for five of the six microclimatic habitats was shorter than the estimates based on DMI temperatures for all pathogens studied. The microclimatic temperatures also predicted a longer season for virus development compared to DMI temperatures. Based on DMI data of hourly temperature, solar radiation, wind speed, rain and humidity, we were able to predict the microclimatic temperature of different habitats with an R^2 of 0.87–0.96. Using only meteorological temperatures for vector-borne disease transmission models may substantially underestimate both the daily potential for virus development and the duration of the potential transmission season.

Temperature is a key driver of vector-borne disease transmission, as replication of arboviruses and parasites within the cold-blooded vectors are dependent on the environmental temperature[1,2]. The extrinsic incubation period (EIP; the time interval between ingestion of the infected blood meals and the ability of the vectors to transmit the virus) and the blood meal digestion period (the time required to complete the cycle from blood feeding to egg-laying and blood feeding again) are greatly influenced by the actual temperature surrounding the vectors[3,4]. Vector-borne disease transmission models for estimating the EIP and blood meal digestion period often use temperatures recorded by a meteorological institute[4–7] rather than the actual microclimatic temperatures to which the vectors are exposed. The use of microclimatic temperatures in vector-borne disease transmission models has been hindered by the lack of microclimatic data.

In the present study, we define meteorological temperatures as temperatures measured at weather stations adhering to the standards given by the World Meteorological Organization (WMO), as described by the Commission for Instruments and Methods of Observation (CIMO)[8]. According to these standards, a WMO site should be representative of a large area (i.e. 100 to 1000 km^2)[8], and the weather station is therefore positioned far from obstacles and complex or urban terrain. To obtain a uniform temperature, recordings are made at specific heights (usually 2 m above the ground), using a white box, ensuring that the thermometer sensor is never exposed to direct sunlight. The standard meteorological temperature is therefore only one of many microclimatic temperatures within an area, and as a result, meteorological parameters observed at a WMO station cannot be fully representative of the potential geographically localised microclimate (e.g. an insect habitat). Using meteorological temperatures in vector-borne disease transmission models ignores the impact of the actual temperature in the habitat to which the insects are exposed.

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The concept of microclimatic temperature was first described in the early nineteenth century by German plant ecologist Gregor Kraus who found that microclimatic temperature is different than meteorological temperature and difficult to formulate[9]. He defined microclimatic temperature as the climate of small space. Another German meteorologist Rudolf Geiger (1950) described the theoretical microclimatic temperature of different locations around the world focusing on urban habitats and habitats such as deserts, snow and forests[9]. Rudolf Geiger (1950) also described the mechanisms of heat exchange at ground surface, the warming and cooling process and relationship between ground surface temperature, and the relationship between ground surface temperature and factors affecting this temperature such as humidity, wind, and solar radiation[9]. Earlier this century, a number of articles published on microclimatic temperature[10–15] but only a few of them were on vector-borne diseases[16,17]. Microclimatic temperatures of Scandinavian countries are largely unknown and so is their potential role in vector-borne disease transmission.

Models used for estimation of vector-borne disease transmission are mostly based on monthly or daily average temperatures[4,5,18]. In reality, the vectors do not experience an "average temperature", but are instead exposed to changing temperatures throughout the day[6]. There is a threshold temperature for most vector-borne diseases, below which a pathogen will not continue to develop in the vector. This threshold is 10.4 °C for bluetongue, 13.3 °C for Schmallenberg virus, 14 °C for Dirofilaria, 14.3 °C for West Nile virus, 15.4 °C for malaria, and 19 °C for dengue virus[4–6,19–21]. Above these threshold temperatures, the rate of virus development and blood meal digestion varies greatly. Some pathogens develop at a higher rate immediately after the threshold temperature is exceeded, while some pathogens develop more quickly at even higher temperatures. Furthermore, some pathogens stop developing above certain temperatures (e.g. 34.4 °C for malaria; see graph S4)[16]. Using daily or monthly average temperatures therefore ignores the effects of threshold temperatures and the non-linear rate of development at increasing temperatures above the threshold values.

In cool Scandinavian climates like in Denmark, the models using monthly or daily mean temperatures could considerably underestimate the potential for vector-borne disease transmission. In Denmark, only two months of the year (July-August) have mean temperatures above the threshold for virus development[22]. However, the country experiences many hours above the threshold temperatures of viral replication in spring (May-June) and autumn (September-October), which is not apparent from the daily or monthly mean temperatures[22].

Insects experience temperature that can be very local down to at a scale of centimeters to meters, yet most vector-borne disease transmission parameters (for example the EIP and blood meal digestion period) are analysed based on meteorological temperatures collected several kilometers away from their habitats[23]. This is because microclimatic temperature data are generally lacking, and the practical difficulties in getting microclimatic temperatures from many different habitats and heights. Studies are available on microclimatic temperatures[10–12,17,23], but only some of these describe the difference between weather-station-based meteorological temperatures and microclimatic temperatures[11,17,23]. In these studies air temperature, humidity, solar radiation and precipitation were identified as key parameters for predicting microclimatic temperature[11,17,23]. Kearney *et al.* published a dataset on predicted microclimatic temperatures of three substrates (soil, rock, and sand) on a global level[10]. Such models or data are not available for terrestrial temperatures, particularly for potential insect habitats applicable to vector-borne diseases. In this study, we modelled microclimatic temperatures of six different habitats in Denmark (dry meadow, wet meadow, hedges, forest/trees, cattle grazing field and horse grazing field) based on available hourly meteorological data from the DMI, measured at weather stations adhering to WMO standards.

Denmark's climate was previously predicted to be too cold for the spread of bluetongue virus[4], yet the country experienced a substantial bluetongue outbreak in the years 2007 and 2008[24]. In this study, we modelled the EIP and blood meal digestion period using microclimatic temperature instead of the traditional approach of using temperatures recorded by meteorological institutes, and used hourly temperature data instead of monthly or daily mean temperatures. Our objectives were to: 1) quantify the difference between meteorological temperatures and actual microclimatic temperatures recorded in potential habitats of biting midges (*Culicoides*) and mosquitoes (*Anopheles*, *Culex* and *Aedes*); 2) compare the seasonal impacts of microclimatic and meteorological temperatures on the development time of bluetongue, Schmallenberg, West Nile and dengue virus, vivax malaria and Dirofilaria parasites and blood meal digestion period of biting midges and mosquitoes; 3) develop a model to predict the hourly microclimatic temperatures of a habitat, based on available parameters from meteorological institutes.

Results

Microclimatic vs. meteorological temperature. We observed large variations between temperatures recorded at six microclimatic habitats at three different heights, and the closest DMI weather station (Table 1). Although there were relatively small differences in the average monthly mean temperatures between DMI records and the different microclimatic habitats (for example 1.0 to 1.3 °C in dry meadow in May 2015), the variation in temperature at specific times was high (for example, −5.8 to 11.4 °C in dry meadow in May 2015), as reflected in hourly differences (5% and 95% percentiles) of microclimatic temperature (Table 1). Overall, the horse and cattle fields had higher monthly mean temperatures (range: 0.01 & 3.2 °C) at all heights (10 to 110 cm) during the entire season (June to October). The upper and mid-height of dry meadow and hedge habitats had higher monthly mean temperatures (range: 0.30 & 1.6 °C) compared to weather stations (except for October). Forest/trees had similar hourly temperatures to DMI records throughout almost the entire study period (maximum difference 0.57 °C). However, the wet meadow was cooler (range −0.07 & −1.8 °C) than meteorological temperatures in late summer and autumn.

We generated frequency distributions of hourly temperatures of microclimatic habitats and weather stations (Fig. 1) and found large variations in the microclimatic habitats, which were both warmer and colder than the temperatures from DMI. We compared the number of hours during which temperatures were above 13.3 °C (the threshold temperature for Schmallenberg virus replication in *Culicoides*[5]) in the microclimatic habitats and DMI

Locations	Heights	No. of loggers	Mean Temp °C (5–95% percentiles)					
			May	June	July	August	September	October
DMI (Strødam) (5–95% percentiles) (°C)	2.5 m		**10.2** (4.7–14.9)	**13.4** (8.3–19.2)	**16.4** (10.4–24.5)	**17.6** (11.2–23.6)	**13.4** (7.1–17.8)	**9.3** (5.1–12.8)
Strødam, Denmark, monthly mean of the hourly difference (5–95% percentiles)								
Dry meadow (°C) – DMI (°C) (5–95% percentiles)	Upper (1.1 m)	6	**1.0** (−2.9–7.2)	**1.2** (−2.9–7.3)	**1.5** (−3.6–8.6)	**1.3** (−3.9–9.1)	**0.60** (−3.7–8.8)	**0.35** (−3.8–5.8)
	Mid-height (0.55 m)	6	**1.3** (−3.5–8.5)	**1.6** (−5.0–9.2)	**1.6** (−5.7–10.8)	**0.80** (−5.5–9.7)	**0.30** (−6.2–7.4)	**−0.90** (− 5.7–4.1)
	Lower (10 cm)	6	**1.1** (−5.8–11.4)	**1.5** (−5.7–11.8)	**0.85** (−5.7–10.8)	**−0.10** (−5.8–9.8)	**−1.1** (−6.6–6.0)	**−1.4** (−6.1–2.0)
Hedges (°C) –DMI (°C) (5–95% percentiles)	Upper (3.3 m)	6	**1.2** (−2.3–7.0)	**1.7** (−2.3–8.7)	**1.6** (−2.1–8.0)	**0.90** (−2.9–6.5)	**0.35** (−2.6–5.0)	**−0.35** (−2.6–2.6)
	Mid-height (2.2 m)	6	**1.2** (−2.2–7.2)	**1.4** (−2.2–7.8)	**1.2** (−2.3–7.2)	**0.75** (−2.6–5.3)	**0.20** (−2.7–4.8)	**−0.40** (−2.6–1.9)
	Lower (10 cm)	6	**−0.15** (−3.7–4.7)	**−0.75** (−3.9–2.4)	**−1.2** (−4.2–1.5)	**−1.3** (−4.5–1.9)	**−1.5** (−5.1–1.0)	**−1.5** (−4.8–0.6)
Tree (°C) –DMI (°C) (5–95% percentiles)	Upper (9.4 m)	2	**0.10** (−2.2–2.4)	**0.12** (−2.3–2.4)	**−0.02** (−2.9–2.3)	**−0.06** (−3.0–2.6)	**0.10** (−2.5–2.5)	**0.07** (−1.7–2.6)
	Mid-height (6.8 m)	2	**0.30** (−2.2–3.0)	**−0.01** (−2.5–2.2)	**−0.13** (−3.6–2.2)	**−0.16** (−3.1–2.3)	**−0.15** (−2.7–2.2)	**0.57** (−0.3–2.0)
	Lower (3.0)	2	**0.47** (−2.1–3.3)	**0.01** (−2.7–2.2)	**−0.15** (−3.6–2.1)	**−0.20** (−3.3–2.4)	**−0.19** (−2.7–2.2)	**0.06** (−1.8–2.4)
Wet meadow (°C) – DMI (°C) (5–95% percentiles)	Upper (0.55 m)	6	**0.18** (−3.5–5.1)	**0.04** (−3.2–3.2)	**−0.65** (−4.1–20.0)	**−1.5** (−4.8–1.0)	**−1.2** (−4.3–1.0)	**−1.16** (−3.4–0.6)
	Lower (0.1 m)	6	**0.42** (−3.3–5.8)	**−0.07** (−3.7–3.2)	**−0.77** (−4.6–2.3)	**−1.8** (−5.2–1.0)	**−1.3** (−4.3–1.1)	**−1.1** (−3.3–0.7)
Faxe, Denmark, (5–95% percentiles)								
DMI (Faxe) (5–95% percentiles) (°C)	2.5 m		**10.0** 5.3–14.1)	**13.1** (7.3–18.8)	**16.1** (10.3–22.7)	**17.6** (11.5–22.8)	**13.3** (7.5–17.2)	**9.3** (5.8–13.3)
Faxe, Denmark, monthly mean of the hourly difference (5–95% percentiles)								
Cattle Field (°C) – DMI (°C) (5–95% percentiles)	Upper (1.2 m)	2	**NA**	**1.9** (−1.8–7.6)	**2.3** (−1.7–8.5)	**1.4** (−2.4–7.1)	**1.2** (−2.3–7.3)	**0.30** (−2.3–3.9)
	Mid-height (0.60 m)	2	**NA**	**2.3** (−1.5–7.9)	**2.3** (−1.4–8.2)	**1.3** (−2.2–6.6)	**1.8** (−2.1–9.3)	**0.86** (−1.9–5.5)
	Lower (0.10)	2	**NA**	**1.2** (−2.0–4.6)	**0.64** (−2.6–4.3)	**0.38** (−3.5–6.2)	**1.3** (−3.5–10.5)	**0.01** (−3.2–3.2)
Horse Field (°C) – DMI (°C) (5–95% percentiles)	Upper (1.2 m)	2	**NA**	**2.3** (−1.2–7.5)	**2.5** (−1.3–8.3)	**1.02** (−3.0–6.2)	**1.3** (−2.2–9.6)	**0.64** (−1.8–7.1)
	Mid-height (0.60 m)	2	**NA**	**2.5** (−1.4–8.1)	**2.9** (−1.5–11.0)	**1.4** (−3.1–6.6)	**1.5** (−2.4–10.9)	**0.38** (−2.1–5.4)
	Lower (0.10)	2	**NA**	**3.2** (−1.5–10.9)	**2.9** (−2.3–11.2)	**2.1** (−4.1–12.5)	**1.1** (−3.0–9.4)	**0.10** (−2.9–4.9)

Table 1. The temperature recorded by the Danish meteorological Institute (DMI) and mean of the difference (5–95% percentiles) between microclimatic data logger temperatures and DMI temperatures at four different habitats in Denmark, May – October 2015. Data from cattle and horse fields were available from 4th June - 31st October 2015, whereas data from other habitats were available from 1st May–31st October 2015.

records, and found that the microclimatic habitats had experienced more hours with temperatures above 13.3 °C ($p < 0.05$), except in wet meadow (July to October; Fig. 1). The microclimatic habitats (especially dry meadow, hedges and cattle and horse fields) were above the threshold temperature for many hours in spring and autumn. In contrast, the wet meadow had fewer hours above 13.3 °C (Fig. 1).

Microclimatic habitats were on average 3.5 to 5 °C warmer than DMI temperatures during midday, and 1 to 3 °C cooler during midnight (Fig. 2). In May, dry meadow (upper height) had mean hourly temperatures >13.3 °C for 10 hours during the day, whereas DMI temperatures did not record a single hour of the day above that temperature ($p < 0.001$; Fig. 2). During spring (May to June), the lower height of the microclimatic habitats had high temperatures during the day and low temperatures during night compared to the upper and mid-height of the habitats. During summer and autumn, the upper height of the habitats had high temperatures compared to the lower height. The difference in hourly temperature between the upper and lower height of the habitats in dry meadow was: −0.10 °C in May, −0.26 °C in June, 0.66 °C in July, 1.4 °C in August, 1.6 °C in September and 1.1 °C in October (Table 1). The temperatures of the upper height of dry meadow were 3 to 5 °C higher during the day when solar radiation was >100 W/m^2, and 1 to 2.5 °C lower during the night when solar radiation was zero, compared to the DMI temperatures. Similar patterns were also observed in other microclimatic habitats.

Extrinsic incubation period (EIP). Different pathogens have different EIP durations in different microclimatic habitats (Fig. 3). Overall, all the pathogens showed faster development using the microclimatic temperatures in dry meadow, hedges, and cattle and horse fields compared to DMI temperatures. Schmallenberg virus, for example, could complete development at a mean of 24.4 days with DMI temperatures, 13.3 days with cattle field temperatures, 13.4 days with horse field temperatures, 15.2 days with dry meadow temperatures, 15.7 days with hedge temperatures, 24.1 days with forest/tree temperatures and 25.5 days with wet meadow temperatures between 4th May and 21st August (the time period during which all habitats allowed for viruses to develop fully). For most of the pathogens, the microclimatic habitats allowed for a longer transmission season compared to DMI temperatures (Fig. 3). For example, the model using DMI data showed that bluetongue virus could only successfully develop in vectors for 92 days after infection, from 17th May to 16th August, with a mean of 36 days. For microclimatic temperatures in the upper height of dry meadow habitat, virus development was possible for 117 days starting from 1st May to 27th August, with a mean of 27 days. Between 4th May and 21st August (when

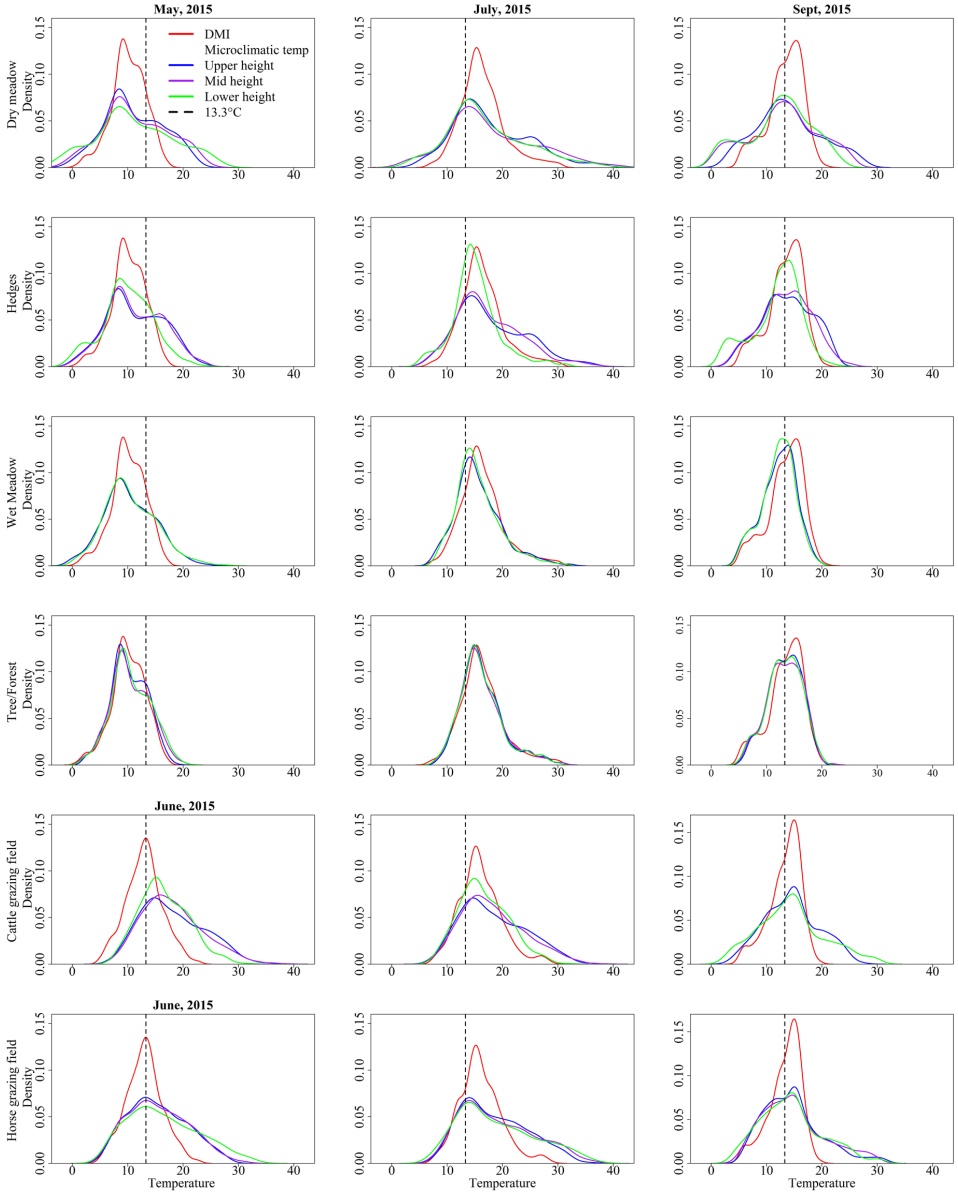

Figure 1. Hourly temperatures measured at six different microclimatic habitats and from the weather stations of the Danish Meteorological Institute (DMI). For illustrative purposes, only the months May, (June, for horse and cattle fields), July and September are depicted. The vertical (black dashed) line is the threshold temperature for pathogen development (Schmallenberg virus, 13.3 °C). Data from cattle and horse fields were available from 4th June - 31st October 2015, whereas data from other habitats were available from 1st May –31st October 2015. The temperature variation was considerably higher in microclimatic habitats, and differences were more evident in spring (May or June) and autumn (September). The dry meadow, horse field and cattle field were warmer than other habitats.

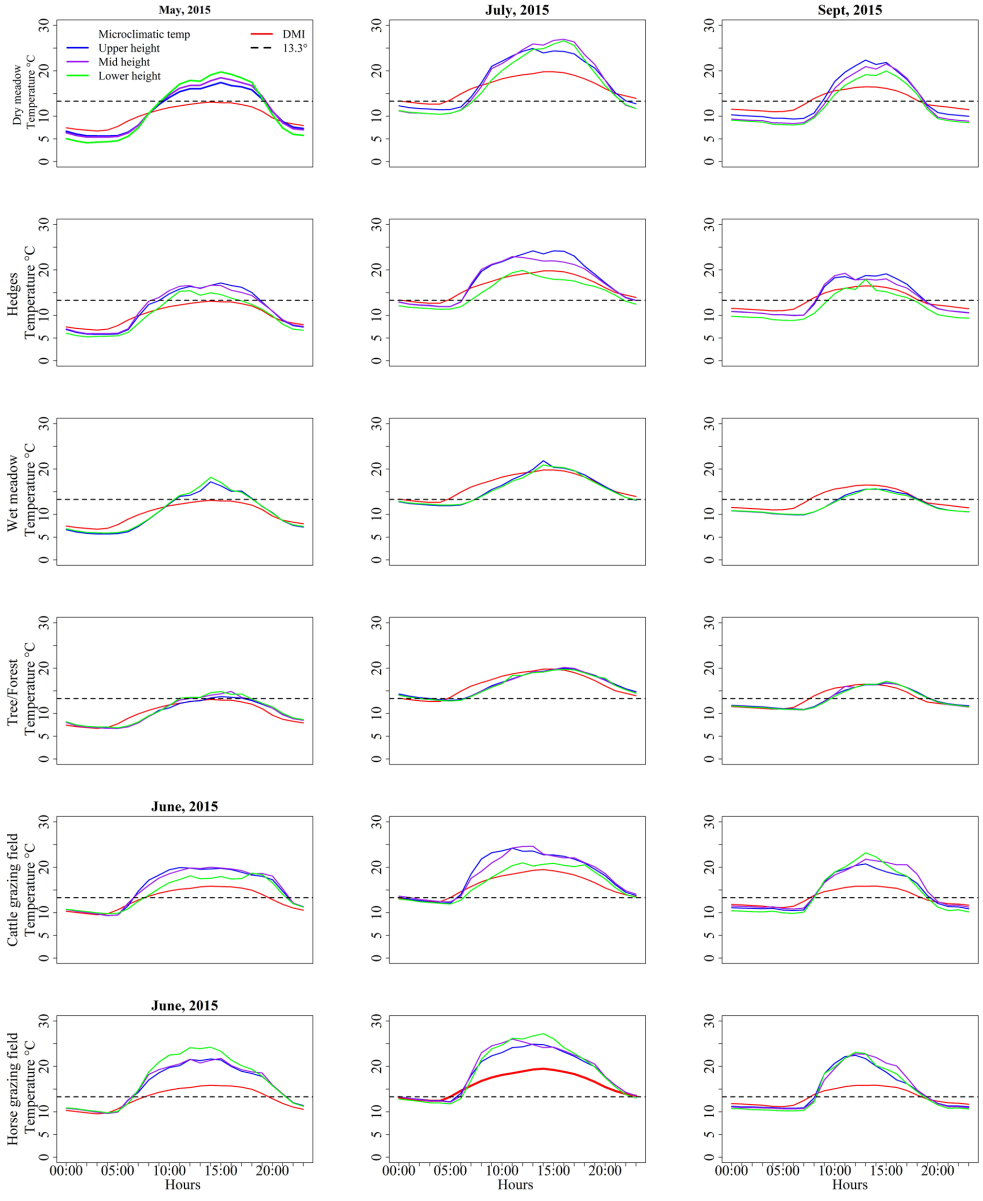

Figure 2. The daily temperature variation at different microclimatic habitat heights and from the weather stations of the Danish Meteorological Institute (DMI). For illustrative purposes, only the months May, June (for the horse and cattle fields), July and September are depicted, with the y-axis showing the average temperature for the month at the time of day given on the x-axis. The horizontal (black dashed) line is the threshold temperature for pathogen development (Schmallenberg virus, 13.3 °C). Data from cattle and horse fields were available from 4th June - 31st October 2015, whereas data from other habitats were available from 1st May – 31st October 2015. Microclimatic habitats were cold at night and warm during the day, and the difference between microclimatic temperatures and DMI temperatures was more evident in the spring (May or June) and autumn (September). The dry meadow, horse field and cattle field were warmer than other habitats.

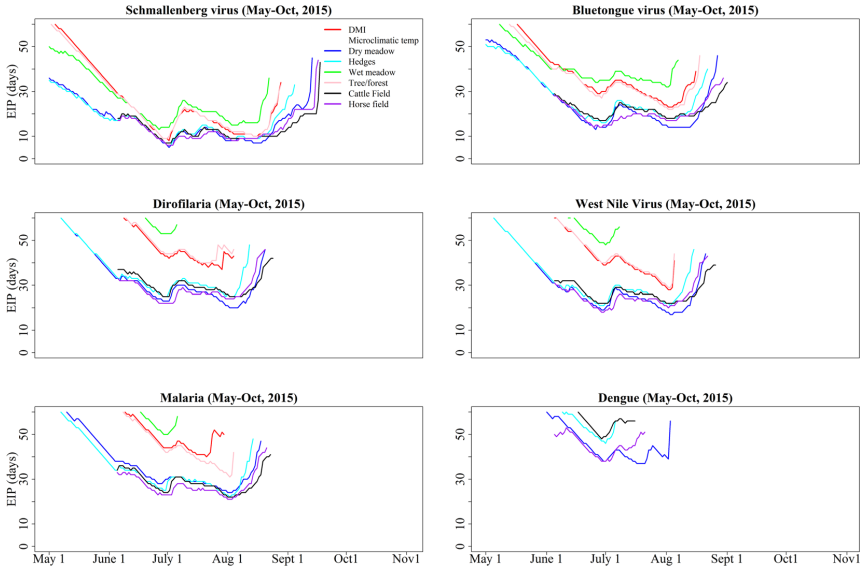

Figure 3. The extrinsic incubation period (EIP) of bluetongue, Schmallenberg, dengue and West Nile virus, Dirofilaria and *Plasmodium sp* (malaria) calculated from temperatures in different microclimatic habitats and from the Danish Meteorological Institution (DMI), May - October 2015, Denmark. Data from cattle and horse fields were available from 4th June - 31st October 2015, whereas data from other habitats were available from 1st May – 31st October 2015. An extended period and faster virus development were observed at microclimatic habitats than for DMI temperatures. Dengue virus did not develop fully when modelled with DMI data.

all habitat temperatures facilitated the development of bluetongue virus) the minimum EIP for DMI data was 23 days, compared to 13 days for dry meadow temperatures (Fig. 3).

The blood meal digestion period. The average number of hours required for complete digestion of a blood meal in biting midges was 258 hours with DMI temperatures, 207 hours with dry meadow, 214 hours with hedges, 280 hours with wet meadow and 252 hours with forest/tree temperatures (1st May to 2nd October). It took on average 258 hours for *Anopheles* mosquitoes to complete blood meal digestion for the DMI temperatures, 167 hours for dry meadow, 169 hours for hedges, 235 hours for wet meadow and 224 hours for forest/tree temperatures (1st May to 18th September; Fig. 4).

The microclimatic temperature model. We performed multiple linear regressions to express the microclimatic temperature of the six different habitats using standard climatic variables from DMI. The six models were able to explain 87 to 96% of the variation in the habitats (Table S1), where the DMI temperature, the temperature of the previous hour, solar radiation, wind speed, humidity, precipitation, months and hours of the day were all important variables in predicting the hourly microclimatic temperature (Figure S2). We evaluated these key drivers of the model in order to understand the role they played in predicting microclimatic temperature. When exploring a variable, we kept all other variables fixed at the median value, and tested the minimum, first quantile, median, third quantile and maximum values of the variable in question. These evaluations showed that microclimatic temperatures increased with increasing solar radiation. For example, on a sunny day, compared to DMI temperature, microclimate habitats were 0.5 to 3.5 °C warmer when solar radiation was 200 (W/m^2), and 4 to 10.5 °C higher when solar radiation was 800 (W/m^2) (Figure S2). High values of precipitation and humidity negatively affected the microclimatic temperature (with the exception of precipitation in hedges). As the precipitation and humidity increased, the difference between microclimatic and DMI temperature decreased. Wind speed played different roles in the different microclimatic habitats (Table S1). For example, the microclimatic temperature in forest/trees and cattle fields dropped as the wind speed increased, yet in other habitats, the wind speed seemed to marginally increase the microclimatic temperature. Validation of the model is described in supplementary document S3 (Model Validation).

Discussion

Despite the cool Scandinavian climate in Denmark, there have been outbreaks of vector-borne diseases in recent times[24]. Microclimatic temperatures may help us understand and quantify the potential for transmission of vector-borne diseases in Northern Europe. We found only a modest discrepancy between the daily mean

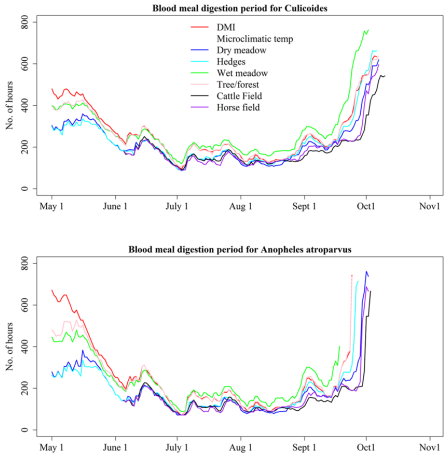

Figure 4. The blood meal digestion period of biting midges (*Culicoides*) and mosquitoes (*Anopheles atroparvus*) calculated from temperatures in different microclimatic habitats and from the Danish Meteorological Institution (DMI), May – October 2015, Denmark. Data from cattle and horse fields were available from 4th June – 31st October 2015, whereas data from other habitats were available from 1st May – 31st October 2015. A maximum of 60 days was assumed for the blood meal digestion in *Culicoides* and mosquitoes. The blood meal digestion was faster in microclimatic habitats than DMI data, and the difference was more evident in the spring (May) and autumn (October).

temperatures from the national meteorological institute data and our recordings in six microhabitats. We did, however, identify a much larger daily variation in microclimatic temperatures compared to national meteorological temperatures, which resulted in higher maximum temperatures at most microclimatic habitats.

The microclimatic habitats (particularly the dry meadow, hedges and cattle and horse fields) were generally warmer than DMI temperatures - possibly due to the direct exposure to sunlight. The agreement between forest/tree temperatures and DMI temperatures may be due to the similar height of the microclimatic temperature loggers and the DMI weather stations, or because of the high exposure to wind. The lack of direct sunlight in wet meadow habitats resulted in a cooler microclimate than the DMI temperatures. Seasonally, temperature variation differed considerably at different heights above the ground, probably due to the growth of grass vegetation during the summer. During the early period of the warm season (spring), the lower heights of the habitats were warmer than the upper and mid-height whereas during late summer and autumn, the temperature at the lower habitats became cooler as the vegetation grew and blocked the sunlight. Microclimatic temperatures are therefore complex and very dependent on local factors such as seasonal vegetation growth.

A number of studies are available on microclimatic temperatures, but most of them concentrated on habitats different from those in our study[9–11,13,15]. Examples include forest microclimates[9,12], underground temperatures[9,10], sea temperatures[14], mosquito breeding habitats (water)[11] and tropical climates[16] – none of which are comparable to our study. One study in tropical settings in Chennai, India, found microclimatic habitats were warmer at night, in contrast to our findings[16]. The study in Chennai was conducted in densely packed urban environments described as 'urban heat islands', which is in stark contrast to our rural temperature data collection sites[25]. Another study looked at seasonal variation in microclimatic conditions in domestic and peri-domestic habitats in rural Argentina and found that microhabitats were generally 5.0 to 5.6 °C higher compared to the ambient temperature[17]. However, we found that the difference between microclimatic and meteorological temperature was mostly in the hourly distributions and only to a lesser extent in daily mean or median temperatures, since microclimatic habitats become warmer during the day and cooler during the night than the meteorological temperature. Temperatures below the threshold have no impact on virus development or the blood meal digestion period. Therefore, daily or monthly mean temperature could mask the hourly variations of temperatures in the model and risk either underestimation (when the mean temperature is below threshold) or over-estimation (when the mean temperature is above threshold) of virus development and blood meal digestion period. Since microclimatic habitats had more hours above the threshold temperature, the impact of microclimatic temperature on the virus development rate and blood meal digestion was noticeable. Microclimatic temperature is therefore very important, especially for countries like Denmark, where daily and monthly average temperatures are often close to the pathogen-development threshold (10 to 15 °C).

Based on our EIP estimates for vector-borne pathogens of the year 2015, we found two important differences between models using meteorological and microclimatic temperatures for estimating the transmission intensity: 1) pathogens develop at a faster rate in microclimatic habitats (except wet meadow) and 2) there is a longer season for vector-borne disease transmission when modelling with microclimatic temperatures compared to meteorological institute temperatures. The increased duration of the pathogen-development season in the vector is an

important finding. Using the microclimatic temperatures of 2015, we found that complete virus development was possible in a period of at least 2.5 months for dengue, 4 months for malaria, 4.5 months for Dirofilaria and West Nile Virus, and 5 months for bluetongue and Schmallenberg virus. In recent years, Schmallenberg virus has been detected in late autumn (September) in Denmark and other European countries[26]. When using meteorological temperature in our transmission model, the Schmallenberg virus did not develop after mid-August in 2015. However, when the observed microclimatic temperature was used in the same model, virus development was possible for one additional month. Therefore, the microclimatic temperature increased both the daily transmission rates and the number of days where transmission could take place, potentially leading to a dramatic change in the accumulated number of cases at the end of the season with an outbreak starting early in summer. We started collecting microclimatic data from May (in dry meadow, wet meadow, hedges, and forest/trees) and June (in cattle and horse fields), but our model shows that virus development and blood meal digestion might occur even earlier in the year. However, it is important to note that although virus development rate is faster at higher temperatures, the vector survival rate is correspondingly lower.

While standard temperatures are available from national meteorological offices for any site in Europe, hourly microclimatic temperatures are not. Our models enabled us to predict the temperature of different microclimatic habitats based on available standard variables from a meteorological institute. The important meteorological variables in our model (i.e. meteorological temperature, solar radiation, humidity, precipitations and wind speed) have also been identified as key variables for predicting microclimatic temperature in previous studies[10,13].

The temperature during the previous hour also played an important role in predicting microclimatic temperature, indicating that it takes time for the temperature in a microclimatic habitat to change. For example, if solar radiation heats up the vegetation and soil in the habitats, it will take time to cool down, while the standard meteorological temperature 2 m above ground can change much more rapidly. In addition, a correlation was found between wind speed and solar radiation. During the day when solar radiation was higher, the wind speed increased, and the opposite was observed at night. We found relative humidity was more important than precipitation for predicting microclimatic temperature (see Table S1), though this might be because there were only a few days with rain during our study period. Our models included the month of the study period as a categorical variable, and predicted microclimatic temperatures therefore differed from month to month, despite all other input variables being the same. This suggests that the models are only useful for habitats and climates that resemble the Danish pattern of vegetation growth and climate seasonality (e.g. northern Europe and southern Scandinavia). In general, the model was good at predicting the microclimatic temperature, but the predictions underestimated the temperature compared to observed microclimatic temperature when there was heavy rain. Wind speed in the dry meadow, hedges and horse field were positively correlated with microclimatic temperature. We selected dry meadow, wet meadow and hedges from a natural reserve area encircled by large trees, so wind speed was obstructed by forest and could not change the temperature at the lower and mid-height of dry meadow and hedges habitats. Likewise, the horse fields were also surrounded by trees, and the influence of wind speed might be different in other microhabitats not surrounded by forest.

Our EIP estimates of different pathogens are supported by empirical findings in Denmark. Our estimate shows that bluetongue and Schmallenberg virus transmission is possible in even late autumn. The country experienced bluetongue virus outbreaks in 2007 and 2008[24] and Schmallenberg virus outbreaks in the autumn, 2012[27]. There was a number of outbreaks of malaria in the sixteenth to eighteenth century and as recent as in the nineteenth century[28], with 33 cases reported as late as 1911 in Denmark[29], which supports our findings that the microclimatic temperature was warm enough to allow malarial parasite development in mosquitoes at that time. The vectors for West Nile virus and Dirofilaria have recently been reported in Denmark[30]. Our findings suggest that it is likely that Dirofilaria (minimum EIP- 20 days) and West Nile virus (minimum EIP- 17 days) could be transmitted in Denmark if introduced. No known vectors for dengue are currently reported in Denmark, and based on our estimation, if they are introduced and become infected; they will have to survive a minimum of 45 days to be able to transmit the virus given the observed temperature in 2015 in Denmark.

The resting sites for biting midges and mosquitoes are largely unknown, but different types of mosquitoes and biting midges might rest on different types and heights of vegetation[31–33]. The distribution of mosquitoes and *Culicoides* depends on the distribution of their hosts and the microclimate of their resting habitats[34]. The resting sites of adult *Culicoides* were reported to be dense vegetation, leaf sheaths and bushes[35]. Carpenter (1951) reported adult *Culicoides* resting on ground litter and on the underside of foliage; he found the equally distributed adult *Culicoides* at 7, 23 and 35 feet above ground[36]. Carpenter *et al.* (2008) confirmed the presence of *Culicoides impunctatus* in extremely large numbers on European white birch (*Betula pubescens*), a deciduous tree native and abundant throughout northern Europe[37]. Natural resting sites for mosquitoes of the eight genera including *Anopheles*, *Culex* and *Aedes* were reported by Burkett-Cadena *et al.* (2008). The predominantly natural resting sites of the adult female mosquitoes were small and large tree cavities, understory vegetation, and trash cans[38]. Above 35 °C all *Anopheles* seek even darker and consequently cooler shelter. They also avoid habitats of high temperatures with low humidities[39]. The literature suggests that both mosquitoes and *Culicoides* do not select resting sites randomly, rather they seek favorable microhabitats to rest in and these microhabitats may be related to shade and increased humidity. However, little is known about their resting behavior during the low temperatures experienced in Scandinavia during spring and autumn when *Culicoides* abundance can be high. Biting midges and mosquitoes can quickly move between microhabitats on a farm and may move between resting sites to optimize conditions. We here quantified a range of microclimates related to farms and grazing areas, but there is a need to understand the vector's actual selection of resting microhabitats in order to model pathogen development times and blood meal digestion period in the vectors and to ultimately model transmission potential of these vectors.

We collected microclimatic temperatures from different habitats. Although we do not know where the vectors rest, we believe the range of temperatures obtained from microclimatic habitats at different heights are more relevant for disease transmission than temperatures measured in a box 2 m above ground by a standard weather

station. We do not know which particular microclimatic temperature is most relevant to select as resting site for the different vector species, so instead of single EIP estimates based on a specific microclimate, we suggest the EIP and blood meal digestion period are better estimated as a range based on different microclimates within a given area of interest. If microclimatic temperatures are not available or not convenient to collect, we recommend using the models described here to predict temperatures, providing the settings are similar to the Danish climate.

Successful completion of EIP of a vector-borne disease depends on the life span of the insect. In our model, we considered a life-span window of 60 days for mosquitoes and biting midges. In published literature, the life span of mosquitoes has been described as 24–67 days[40] and for biting midges between 10 to 30 days, but they can survive up to 90 days under very cool weather conditions[41].

Conclusion

Our model shows that viruses develop at a faster rate and have a longer season of transmission when modelled with microclimatic temperature compared to meteorological temperature. The reason for this is that microclimatic temperature differs greatly from meteorological temperature. Microclimatic habitats were warmer during the day and cooler during the night compared to the meteorological temperature, despite relatively small difference in the mean temperatures of both estimates. Using standard meteorological temperatures (especially in a cool temperate climate like in Denmark) can substantially underestimate the potential for vector-borne disease transmission, particularly for zoonotic pathogens like Dirofilaria and West Nile virus, and human pathogens like malaria and dengue. Using daily or monthly mean temperature carries a risk of underestimation or overestimation of vector-borne disease transmission parameters. Although no simple relationship exists between microclimatic temperature and meteorological temperature, it is possible to model a range of microclimatic temperatures based on standard meteorological data and use this range of estimated temperatures to drive vector-borne disease transmission models.

Methods

Study sites. We collected microclimatic and meteorological temperatures at Strødam and Faxe in Denmark. Strødam (N55.96007°, E012.27465°) is a 20-hectare natural reserve area with protected woodlands, meadows and open fields. We chose four nearby habitats with different vegetation types: dry meadow, wet meadow, hedges and woodland (forest/trees) at which to record the microclimatic temperature. We set up a portable weather station to record the temperature and other parameters from the same area in order to identify the important variables for modelling microclimatic temperature that should be requested from DMI. Faxe is a small municipality south of Copenhagen. We recorded the microclimatic temperature from one horse grazing field (N 55.22383°, E012.04494°) and one cattle grazing field (N 55.20258, E012.11440) in the municipality.

Recording Microclimatic temperature. Microclimatic temperatures were recorded using "Temperature logger (21G) $^{(TM)}$" data loggers that record the temperature in a single spot of about 3 square centimeters[42]. The temperature loggers are portable battery-powered instruments that can be deployed anywhere to record the temperature of the surroundings[42]. We recorded temperatures at three different heights from dry meadow, hedges, forest/trees, cattle and horse field, but the vegetation in the wet meadow was only tall enough to allow us to measure at two different heights in this habitat. Dry meadow is grassland where green succulent grass grows during spring, summer and early fall. The vegetation grows up to 1.3–1.5 meters. Wet meadow is a wetland where standing water could be present for a short period of time during the growing period. The vegetation grows up to 0.50–0.75 meters. Hedges are collections of plants (usually shrubs) and other tree species implanted in a way to create a barrier or to mark a boundary usually between two neighboring areas. The shrubs grow up to 3.0–3.5 meters. For forest/tree, we chose a tall deciduous tree (*Prunus sp*) approximately 9.5 meters high in a forest in Strødam. The tree was located deep in the forest surrounded by similar trees. Cattle grazing land were chosen from grazing land near a wheat field in Faxe, Denmark. We recorded the temperature from the grassland close to the electric fence. The grasses grow up to 1.0–1.5 meters. Horse grazing land was chosen from a place where horses were allowed to graze in a field close to a horse stable at Faxe, Denmark. This grassland habitat resembles dry meadow but was surrounded by large trees. We used three different sites for each habitat (except forest/trees, cattle and horse field). For the dry meadow, wet meadow, hedges, cattle and horse field, the temperature loggers were placed from 10 cm above the ground (lower) to the top of the vegetation: 1.1 m for dry meadow, cattle field and horse field, 3.3 m for hedges, and 0.60 m for wet meadow. The mid-height loggers were placed approximately halfway between the upper and lower loggers. In forest/trees, we placed the loggers at 9.4 m (upper), 6.8 m (mid) and 3.0 m (lower). Two temperature loggers were placed in one small transparent plastic bag at each height after removing the air manually. The small plastic bag was used to protect the loggers from being directly exposed to rain. The loggers recorded the temperature every 30 minutes and we downloaded the temperatures to a portable data recorder "TempTec-R"[43] at 15–30-day intervals. In total, there were 54 loggers (18 in dry meadow, 18 in hedges, 12 in wet meadow and 6 in trees) in Strødam and 12 loggers (6 in the horse field and 6 in the cattle field) in Faxe.

Meteorological climatic data collection. Based on our initial analysis from the portable weather station data from Strødam, we identified hourly temperature (° C), solar radiation (W/m^2), humidity (relative humidity, in %), wind speed (m/s, 10 minutes average), and precipitation (mm/hour) as potentially important variables for predicting microclimatic temperature. We then requested those data from DMI from 1st May to 31st October 2015. We received the meteorological data recorded by weather station at Sjælsmark (N 55.94407, E 12.27065), 1.8 km away from the Strødam data collection point, and at Faxe Ladeplads (N55.2076, E 12.09807), 1.3 km away from the cattle field and 4.6 km away from our horse field.

Data analysis. We calculated summary statistics of temperatures recorded from DMI expressed as the mean and 5% and 95% percentiles for each month (May to October 2016; Table 1). We then deducted the hourly DMI

Pathogens	Equations	References
Bluetongue virus	$0.0003 \times T \times (T-10.4057)/24$	4
Schmallenberg virus	$(0.019 \times (T - 13.3))/24$	5
Dengue virus	$(-0.1393 + 0.008T)/24$	21
Dirofilaria immitis	$(T - 14/130)/24$	19, 45
Malaria	$(0.000126 \times T (T-14.244) \times \sqrt{(34.4-T)})/24$	16
West Nile virus	$(-0.132 + 0.0092 \times T)/24$	20

Table 2. The equations used to model the changes in the Extrinsic Incubation Period (EIP) of six different pathogens using temperatures from the Danish Meteorological Institute (DMI) and microclimatic temperatures recorded at Strødam and Faxe, May–October 2015. T = hourly temperature.

temperature from the hourly microclimatic temperature for each height for all six months and reported their mean and 5 and 95% percentiles. Then, we plotted the number of hours of microclimatic and DMI temperature data from each month and habitat (Fig. 1). We plotted the daily variation in temperature at different microclimatic habitat heights and DMI for each month by calculating the monthly average, and then comparing the number of hours $\geq 13.3\,^{\circ}C$ to the DMI temperature (Fig. 2). The summary statistics and graphs were prepared in the statistical software R[44].

Linear regression to predict microclimatic temperature. We extracted hourly temperature, solar radiation, wind speed, precipitation and humidity information from the DMI weather data. These variables were all included in the initial model to express the microclimatic temperature of a particular habitat. In addition, the month and time of day (as categorical variables), and the height of the data loggers were included in the linear regression model. The following steps were then taken:

1. The initial model included eight variables: meteorological temperature, solar radiation, wind speed, precipitation, humidity, time of day (24 hours), month (each month between May and October as a categorical variable) and height of the habitats (as a categorical variable).
2. The day also played a role in our microclimatic models, but it was not practical to consider each day as a categorical variable. The temperature on 31st May is more likely to be influenced by both May and June than just May, so we used a floating weight for each day, giving the 15th day of each month (taken to be the mid-point) a weight of 1, with other days influenced by neighbouring months, as expressed by the formula:

$$\text{Month(x)} = 15 - (\text{absolute(Date)}/\text{No. of days of month})$$

   This is a 30 or 31-day window and the sum of the weight of each day is always 1. To avoid negative values originating beyond these 30 or 31days, all negative weights were set to 0 (Figure S5).
3. Five significant interactions were identified in the initial analysis and were added to the model:
   a. Solar radiation and wind speed
   b. Rain and humidity
   c. Solar radiation and month
   d. Wind speed and height
   e. Solar radiation and height
4. We added a 1-hour lag of DMI temperature ($\text{Temp}_{\text{DMI(t-1)}}$) in the model
5. The final model was:

$$\begin{aligned}(\text{Temp}_{\text{micro}}) = {} & (\text{Temp}_{\text{DMI}}) + (\text{Temp}_{\text{DMI}(t-1)}) + \text{solar radiation} + \text{wind speed} \\ & + \text{humidity} + \text{Month weight (May)} + \text{Month weight (June)} \\ & + \text{Month weight (July)} + \text{Month weight (August)} \\ & + \text{Month weight (September)} + \text{Month weight (October)} \\ & + \text{Time of day} + (\text{solar radiation} * \text{wind speed}) \\ & + (\text{precipitation} * \text{humidity}) + (\text{solar radiation} * \text{month}) \\ & + (\text{wind speed} * \text{height}) + (\text{solar radiation} * \text{height})\end{aligned}$$

where $\text{Temp}_{\text{micro}}$ and Temp_{DMI} are hourly microclimatic and DMI temperatures in Celsius, and height is a categorical value representing the upper, mid-height and lower loggers from the ground.

Calculating the extrinsic incubation period (EIP) of vector-borne diseases. We calculated the EIP of four viral diseases: bluetongue virus, Schmallenberg virus, West Nile and dengue virus, as well as Dirofilaria and the malaria parasite (*Plasmodium vivax*), using the same hourly temperature as described for the microclimatic temperature model (Table 2). We assumed zero pathogen development below the threshold temperature for each pathogen[4,5,19–21]. In addition, we assumed zero growth $\geq 34.4\,^{\circ}C$ for malaria, as suggested by recent studies[6,16]. This is a rate summation model where virus development is calculated hourly and summed up until the virus

Species	Equations	References
Culicoides sp (vector of bluetongue and Schmallenberg virus)	(0.0002 × T × (T–3.7) × (41.9–T)^(1/2.71))/24	4
Anopheles atroparvus (vector of malaria)	((T − 9.9)/36.5)/24	46

Table 3. The equations used to model the changes in the blood meal digestion period of biting midges (*Culicoides*) and mosquitoes using temperature from the Danish Meteorological Institute (DMI) and microclimatic temperature recorded at Strødam and Faxe, May - October 2015. T = hourly temperature.

development is completed (reached a value of 1)[4–6, 19–21, 45] or blood meal digestion[4, 46] is completed. The model was developed in the Statistical Software SAS[47].

Estimating the blood meal digestion period in Culicoides (biting midges) and mosquitoes. We estimated the time (in hours) required for *Culicoides sp* and *Anopheles atroparvus* to digest a blood meal (Table 3), as described elsewhere[4, 46], assuming a 60-day life cycle for both *Culicoides* and mosquitoes. This is a rate summation model where blood meal digestion is calculated for each hour and summed up until the blood meal digestion is completed (reached a value of 1).

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Author Contributions

N.H. led the field implementation of the study, manuscript writing and data analysis, C.K. helped with data analysis and preparation of the graphs, B.K. helped with field set up, L.J.K. helped to interpret the data analysis and provided critical input in manuscript writing, J.H. provided the meteorological data and helped to analyze and interpret the microclimatic and D.M.I. data, R.B. planned the original study, helped with data analysis, developed the E.I.P. and blood meal digestion model and critically reviewed the manuscript. All authors reviewed and approved the manuscript for submission.

Additional Information

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Manuscript 1: Supplementary documents

Microclimatic temperatures increase the potential for vector-borne disease transmission in the Scandinavian climate

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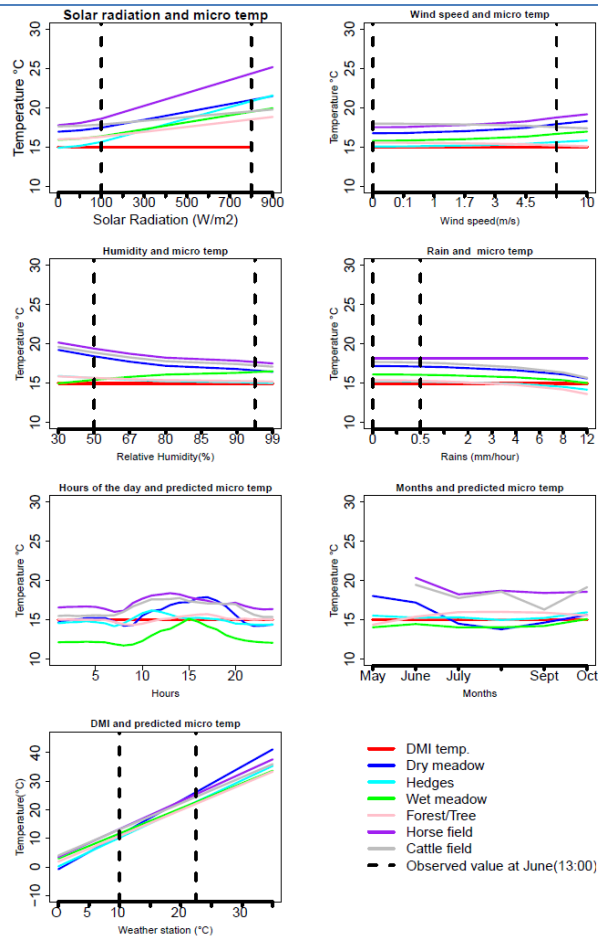
Supplementary figures and tables

Table S1: Co-efficients of multiple linear regression analysis of microclimatic temperatures based on parameters collected from the Danish Meteorological Institute (DMI), May - October 2015. Data from cattle and horse fields were available from 4th June - 31st October 2015, whereas data from other habitats were available from 1st May – 31st October 2015.

Parameters	Dry meadow (ß)	Hedges (ß)	Forest/Trees (ß)	Wet meadow (ß)	Cattle field (ß)	Horse field (ß)
Intercept	-3.29615	-1.291392	3.142008	-0.540491	7.458548	-0.567781
DMI temperature	-0.50749	-0.252880	-0.218472	-0.043402	-0.402979	-0.222878
Previous hour temp	1.704965	1.256425	1.116945	0.920504	1.315854	1.197747
Wind speed	0.17834	0.094738	-0.062509	0.104489	-0.031547	0.194179
Solar radiation	0.006394	0.008497	0.000364	0.003825	0.004614	0.010597
Humidity	-0.04047	-0.013050	-0.009696	0.022252	-0.036530	-0.038442
Rain	-2.56966	-1.421381	-0.784311	-1.090726	-1.262409	-1.136971
Height (lower)	1 (ref)	1	1	1	1	1
Height (mid)	0.424025	1.921676	0.042642	NA	1.175123	0.435755
Height (upper)	1.846005	2.016195	0.162348	-0.041653	0.884637	0.508778
Daily weight (May)	2.212621	-0.354158	-1.194250	-1.710890	-1.656993	4.395879
Daily weight (June)	1.51343	-0.692114	-1.744299	-1.589233	-3.975124	3.008218
Daily weight (July)	4.281054	-0.050915	-2.108724	-0.577108	-1.932351	6.222419
Daily weight (Aug)	4.403061	-0.134437	-2.440829	-1.300479	-3.699297	4.412191
Daily weight (Sept)	4.250463	-0.343676	-2.445684	-1.046149	-1.726114	5.295510
Daily weight (Oct)	4.41985	-0.746235	-2.395448	-2.896761	-4.420269	5.036010
Month (May)	1	1	1	1	NA	NA
Month (June)	-0.83561	-0.258953	1.016326	0.414985	1	1
Month (July)	-3.52574	-0.218181	1.602317	-0.011944	-1.683031	-2.104695
Month (Aug)	-4.23109	-0.557553	1.634379	0.007538	-0.875904	-1.639220
Month (Sept)	-3.38687	-0.301393	1.529616	0.164828	-3.146251	-1.925644
Month (Oct)	-2.41106	0.426661	1.187070	1.042442	-0.297970	-1.777821
Time (00:00)	1	1	1		1	1
Time (01:00)	0.250053	0.109278	-0.027686	0.023701	0.055659	0.077398
Time (02:00)	0.345663	0.137103	-0.064083	0.026198	-0.017128	0.094201
Time (03:00)	0.538556	0.244270	-0.036496	0.075849	0.033391	0.126813
Time (04:00)	0.545741	0.135197	-0.060453	0.029657	0.090918	0.074449

Time (05:00)	0.55761	-0.005559	-0.068613	-0.001704	0.045653	-0.175052
Time (06:00)	0.077547	-0.386145	-0.335666	-0.236922	0.082368	-0.542660
Time (07:00)	-0.42283	-0.012428	-0.641177	-0.409374	0.524860	-0.417640
Time (08:00)	-0.35725	0.623848	-0.714931	-0.280098	1.205719	0.574090
Time (09:00)	0.851712	1.278923	-0.555546	0.156723	1.661110	1.119157
Time (10:00)	1.434443	1.638899	-0.266045	0.860988	2.109240	1.524528
Time (11:00)	1.604866	1.429628	0.011957	1.447743	2.100046	1.664145
Time (12:00)	2.200384	1.104131	0.114004	1.711116	2.150003	1.821707
Time (13:00)	2.517669	0.711100	0.334918	2.321634	2.274665	1.668593
Time (14:00)	2.553804	0.628973	0.470670	2.982250	1.875389	1.317403
Time (15:00)	3.111274	0.697858	0.604793	2.611629	1.723889	1.046330
Time (16:00)	3.196034	0.609259	0.657485	2.029887	1.588638	0.860489
Time (17:00)	2.755565	0.516792	0.430146	1.628588	1.583145	0.519865
Time (18:00)	2.022618	0.301354	0.117382	0.902085	1.515682	0.436791
Time (19:00)	0.938271	-0.035513	-0.012613	0.500101	1.504069	0.603502
Time (20:00)	-0.07934	-0.095565	0.094742	0.230705	0.720162	0.199800
Time (21:00)	-0.46764	-0.173264	-0.040348	0.079239	0.144805	-0.101964
Time (22:00)	-0.41889	-0.198443	0.033351	-0.016014	-0.136991	-0.243449
Time (23:00)	-0.28498	-0.158937	-0.024441	-0.062353	-0.131455	-0.206334
Wind× Month(May)	1	1	1	1	NA	NA
Wind× Month(June)	0.140528	0.090745	-0.042541	0.047163	1	1
Wind× Month(July)	0.088279	-0.098361	-0.022605	-0.042760	0.067163	-0.000569
Wind× Month(Aug)	-0.00095	0.054973	0.067002	-0.136178	0.111277	-0.039945
Wind× Month(Sept)	-0.06374	0.028738	-0.008487	-0.136344	0.266283	-0.172370
Wind × Month(Oct)	-0.22943	-0.102619	-0.016493	-0.172637	-0.073921	-0.170430
Solar radiation × Month(May)	1	1	1	1	NA	NA
Solar radiation × Month(June)	-0.00084	-0.000575	-0.001453	-0.003432	1	1
Solar radiation × Month(July)	0.000068	-0.001064	-0.001990	-0.004728	0.002018	0.001148
Solar radiation × Month(Aug)	6.66E-06	-0.002641	-0.001767	-0.005463	-0.001457	-0.000571
Solar radiation × Month(Sept)	-0.00036	-0.003305	-0.001643	-0.007005	0.006644	0.005799
Solar radiation × Month(Oct)	0.000007	-0.004518	-0.000146	-0.007133	0.000755	0.005474
Rain × Humidity	0.030168	0.016478	0.007892	0.012372	0.013222	0.013732
Wind speed × solar radiation	-0.00045	-0.000368	0.000337	0.000212	-0.000554	-0.000598
Wind speed × height(lower)	1	1	1	1	1	1
Wind speed × height (mid)	0.12571	0.029565	-0.011841	NA	-0.023922	-0.128821
Wind speed × height (upper)	-0.17925	0.029798	-0.046153	0.021786	-0.013419	-0.186326
Adjusted R^2	**0.9043**	**0.903**	**0.961**	**0.904**	**0.871**	**0.891**

Figure S2: The relationship between predicted temperature, solar radiation, wind speed, humidity, precipitation, DMI temperature, hour of the day and month at different microclimatic habitats at 13:00 in June. In the first six graphs, the DMI temperature was fixed at 15^0C and all values were fixed at their respective median, in the last graph DMI temperature varied (from 0 to 35) and all values were fixed at their respective median.



Supplementary document S3: (Model validation)

Method:

Compare observed and predicted temperatures:

To validate the model, we divided the dataset into two equal halves: the first half contained the data of odd week numbers of the study period (week 19 to 43) and the second half contained the even week numbers of the study period (week 18 to 44). We then created a multiple linear regression model using the first dataset (odd week), and used this model to predict the hourly temperatures for the second dataset (even weeks). Finally, we plotted the observed and predicted temperatures of even weeks (Appendix Figure S3.1) and the residuals of the microclimatic models (Appendix S3.2). The dry meadow at Strødam and the horse and cattle fields at Faxe were similar habitats. To compare how our models behaved independently of location, we used the model developed for the dry meadow in Strødam to predict the microclimatic temperature of the cattle and horse fields at Faxe, and compared the observed and predicted temperatures.

Results:

Compare observed and predicted temperatures:

The predicted temperatures could explain 95 - 97% of the variation in the observed temperature in all microclimatic habitats (R^2 = 0.95 - 0.97), and had a correlation coefficient of 0.92 - 0.98 with the observed microclimatic temperature. The residuals from the models were randomly distributed around zero throughout the period (Appendix S3.2). The root mean square error (RMSE) we observed in our microclimatic model was 2.17 for dry meadow, 1.87 for hedges, 0.87 for forest/trees, 1.44 for wet meadow, 2.10 for the cattle field, and 2.20 for the horse field.

The predicted temperature generated by the dry meadow model could explain 85% of the variation in the observed temperatures in the cattle field ($R^2 = 0.85$) and 83% of the variation in the observed temperatures in the horse field ($R^2 = 0.83$; Appendix S3.3).

Figure S3.1: The observed versus predicted microclimatic temperature during even weeks of six habitats (upper height), based on a microclimatic temperature model of odd weeks only, May – October 2015. Data from cattle and horse fields were available from 4th June - 31st October 2015, whereas data from other habitats were available from 1st May – 31st October 2015. The predicted temperature closely matched the observed temperature (correlation coefficient = 0.92 - 0.97).

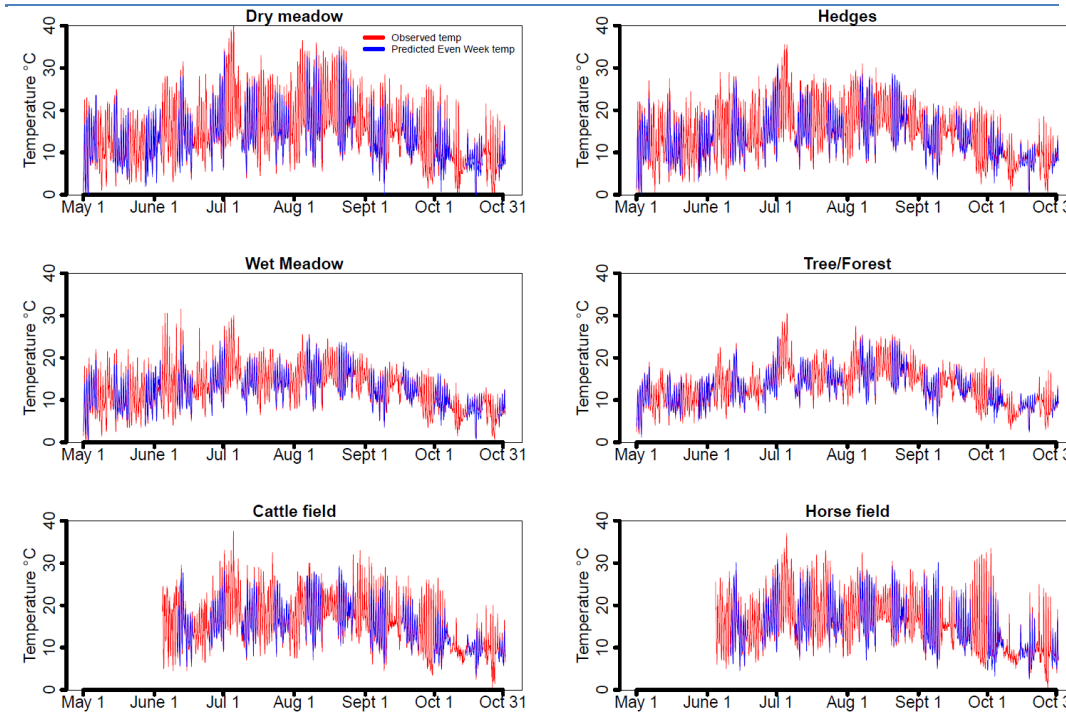


Figure S3.2: The residuals of observed and predicted hourly temperatures of our microclimatic model for dry meadow, hedges, forest/trees, wet meadow and cattle and horse fields, May - October 2015, Denmark. The residuals are randomly distributed around zero.

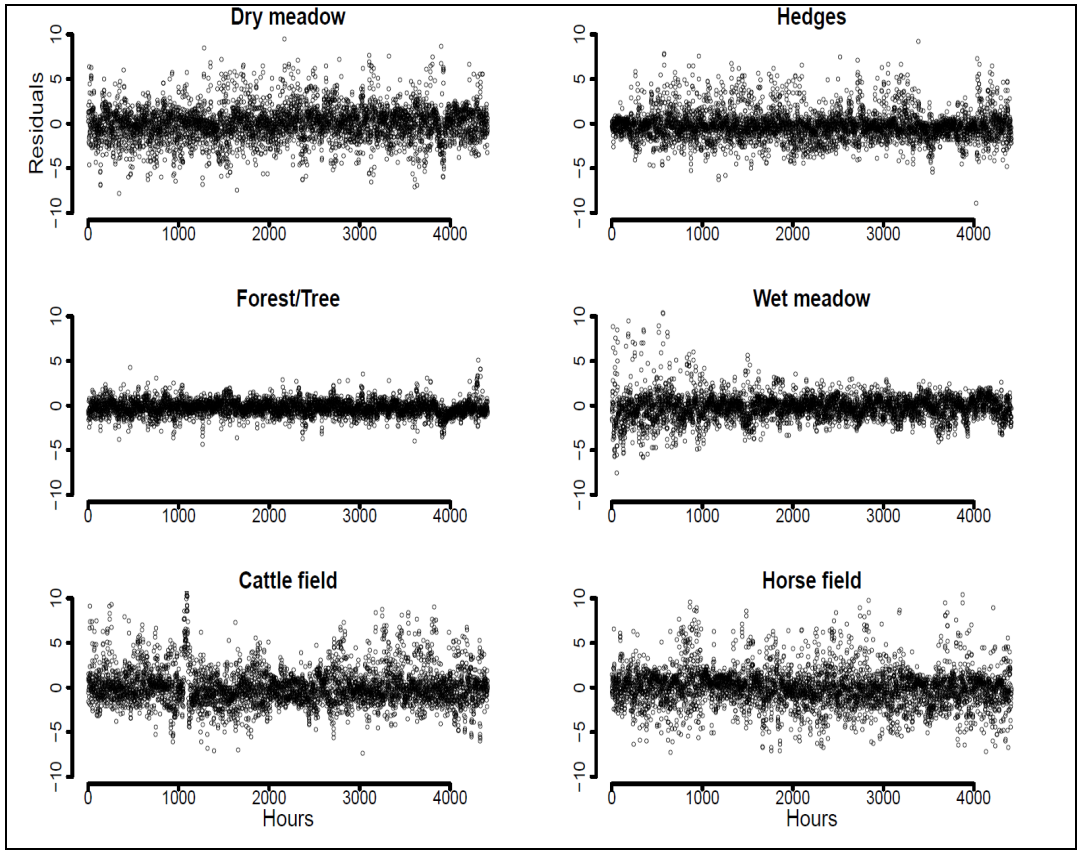


Figure S3.3: Comparison of the observed temperature (Obs.) and predicted microclimatic temperature (Pred.) at cattle and horse fields based on the model of dry meadow at Strødam (from the upper logger), June – October 2015. The predicted temperature at cattle and horse field of Faxe could explain 83% and 85% variation, respectively in the observed temperature in those habitats based on the model of the dry meadow at Strødam, Denmark.

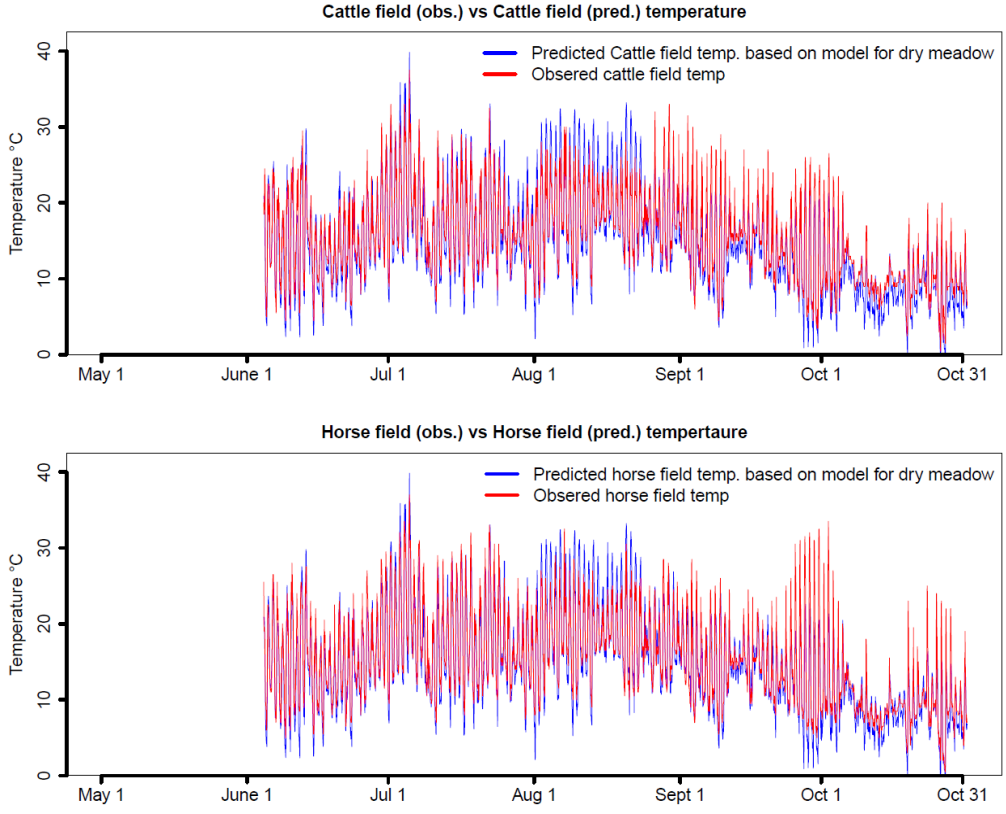
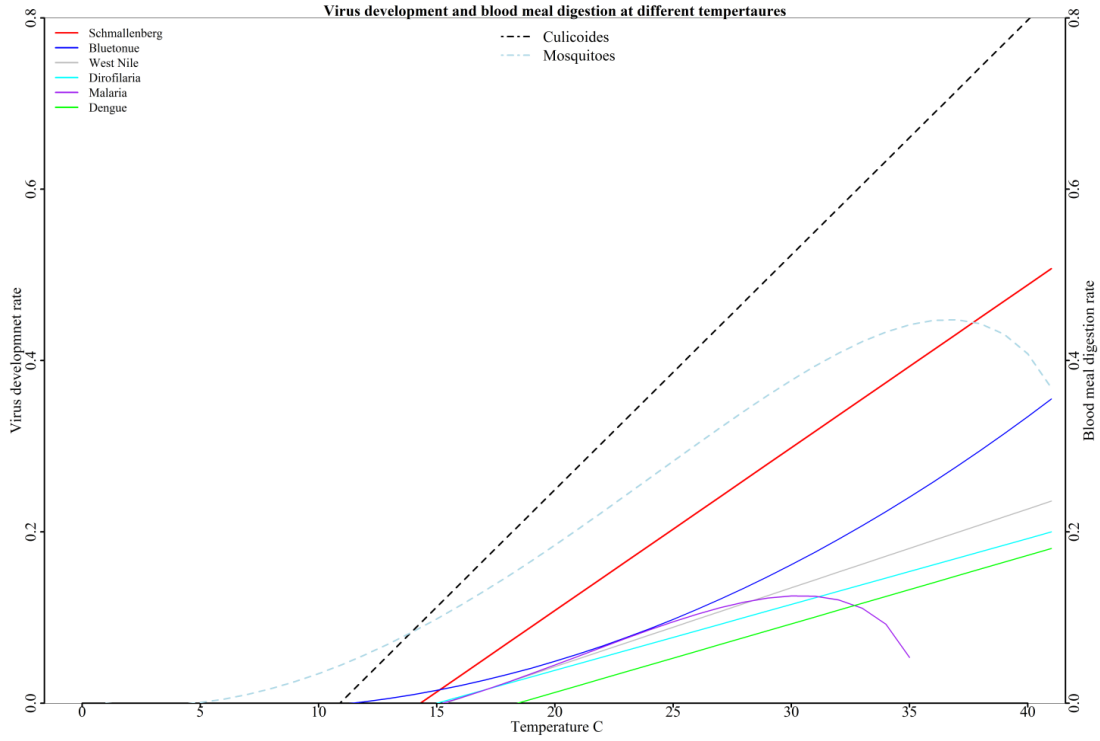


Figure S4: The relationship between temperature, the development rate of pathogens and the blood meal digestion rate in mosquitoes and *Culicoides*. The threshold of pathogen development was between 10^0C (Schmallenberg) and 19^0C (dengue).



Manuscript II

Microclimatic temperatures at Danish cattle farms, 2000–2016: quantifying the temporal and spatial variation in the transmission potential of Schmallenberg virus

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Microclimatic temperatures at Danish cattle farms, 2000–2016: quantifying the temporal and spatial variation in the transmission potential of Schmallenberg virus

Najmul Haider[1*], Ana Carolina Cuellar[1], Lene Jung Kjær[1], Jens Havskov Sørensen[2] and Rene Bødker[1]

Abstract

Background: Microclimatic temperatures provide better estimates of vector-borne disease transmission parameters than standard meteorological temperatures, as the microclimate represent the actual temperatures to which the vectors are exposed. The objectives of this study were to quantify farm-level geographic variations and temporal patterns in the extrinsic incubation period (EIP) of Schmallenberg virus transmitted by *Culicoides* in Denmark through generation of microclimatic temperatures surrounding all Danish cattle farms.

Methods: We calculated the hourly microclimatic temperatures at potential vector-resting sites within a 500 m radius of 22,004 Danish cattle farms for the months April to November from 2000 to 2016. We then modeled the daily EIP of Schmallenberg virus at each farm, assuming vectors choose resting sites either randomly or based on temperatures (warmest or coolest available) every hour. The results of the model output are presented as 17-year averages.

Results: The difference between the warmest and coolest microhabitats at the same farm was on average 3.7 °C (5th and 95th percentiles: 1.0 °C to 7.8 °C). The mean EIP of Schmallenberg virus (5th and 95th percentiles) for all cattle farms during spring, summer, and autumn was: 23 (18–33), 14 (12–18) and 51 (48–55) days, respectively, assuming *Culicoides* select resting sites randomly. These estimated EIP values were considerably shorter than those estimated using standard meteorological temperatures obtained from a numerical weather prediction model for the same periods: 43 (39–52), 21 (17–24) and 57 (55–58) days, respectively. When assuming that vectors actively select the coolest resting sites at a farm, the EIP was 2.3 (range: 1.1 to 4.1) times longer compared to that of the warmest sites at the same farm.

Conclusions: We estimated a wide range of EIP in different microclimatic habitats surrounding Danish cattle farms, stressing the importance of identifying the specific resting sites of vectors when modeling vector-borne disease transmission. We found a large variation in the EIP among different farms, suggesting disease transmission may vary substantially between regions, even within a small country. Our findings could be useful for designing risk-based surveillance, and in the control and prevention of emerging and re-emerging vector-borne diseases.

Keywords: Schmallenberg virus, Microclimatic temperatures, EIP, Spatio-temporal modeling, Denmark, Vector-borne diseases, Transmission, *Culicoides* spp., Resting sites, Cattle farm

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Background

Schmallenberg is an emerging *Culicoides*-borne disease affecting cattle, sheep and goats, and is characterized by pyrexia, reduced milk production, abortion and congenital malformations in the offspring of infected animals [1–3]. Schmallenberg virus was detected in Germany for the first time in November 2011 [1] after which the virus spread to most of the countries in central and northern Europe, including Denmark in 2012 [4]. The virus development rate in insects, known as the extrinsic incubation period (EIP), is the time interval between ingestion of an infected blood meal and the ability to transmit the virus to a new host [5]. The EIP is highly dependent on the temperature surrounding the biting midges [6–8], which is called the microclimatic temperature [9]. The microclimatic temperature of a small geographic area is highly influenced by the presence and intensity of solar radiation, the level of humidity, the speed and direction of the wind, the topography, aspect and local precipitation [9, 10]. These factors are affected by vegetation and land cover, which therefore play an important role in determining the microclimatic temperatures in the available resting sites surrounding a cattle farm [10].

Vector-borne disease transmission models commonly use the temperature recorded by meteorological weather stations [7, 11]. Meteorological temperatures are recorded by weather stations according to the standards set up by the World Meteorological Organization (WMO) [12]. The weather stations are set up at very specific heights all over the world (generally 2 m above the ground), using a specific (white) colored box and placed in a way to protect the thermometer sensor from direct sunlight. As the WMO instructed, a WMO weather station site should be representative of a large area (i.e. 100–1000 km^2) [12]. The area a weather station represents might have a number of different microclimatic habitats and therefore, the standard meteorological temperature does not fully represent all the different climatic condition of insect microhabitats [10, 13, 14]. When these meteorological temperatures are used in vector-borne disease transmission models, the models ignore the real temperature in the microhabitats the insects are actually exposed to [10, 13, 15]. These weather stations may be located as far as 50–100 km from the cattle farms in question and, more importantly, the meteorological temperature recorded by the weather station will only represent one of many potential microclimates of the area. The use of microclimatic temperature in disease modeling is hindered by the lack of data from microclimatic environments [10].

Previous studies have shown that many habitats have warmer microclimatic temperatures than the standard meteorological temperatures [10, 14, 15]. Even when the average daily microclimatic temperature is similar to the average meteorological temperature, the microclimatic temperature is more extreme being relatively warmer during the day and cooler during the night [10, 15]. Virus development in insect vectors is highly dependent on temperature [6, 7], but the relationship is not linear and often shows a threshold temperature, below which virus development is not possible [7, 8, 16]. Therefore, the higher daytime microclimatic temperatures result in average virus development times that are often much shorter than development times at meteorological temperatures [10].

Farm-level microclimatic temperature is not available from registers in Denmark or potentially the rest of Europe. Furthermore, vector-borne disease transmission is rarely assessed at the individual farm level, although a recent study predicted the potential of between-farm transmission of Schmallenberg virus in the UK [17]. The model showed that Schmallenberg virus can infect more farms and spread considerably further than bluetongue virus in the same time frame.

The resting site of an insect refers to the places where the insect rests after taking a blood meal [18, 19]. *Culicoides* spend almost 90% of their life time resting during which they develop oocysts to the appropriate stage for acquiring a blood meal, digestion of the blood meal and developing eggs [18].

The resting sites of biting midges in Denmark are largely unknown, but different species of biting midges may prefer different types and heights of vegetation [20]. A study in the Netherlands found *Culicoides* spp. in wetlands, peat bogs, riverine areas and livestock farms, but higher numbers of biting midges were recorded in wetland areas and peat bogs [15]. Carpenter et al. [21] found *Culicoides impunctatus* in very high numbers on European white birch (*Betula pubescens*), a deciduous tree native to northern Europe. Carpenter [22] reported adult *Culicoides* spp. resting on ground litter and on the underside of foliage. He also found adult *Culicoides* spp. equally distributed at 2.2, 7.0 and 10.7 m above ground [22]. Biting midges seek favorable microhabitats, and their choice is driven by temperature and humidity [22, 23]. However, little is known about their resting behavior at low Scandinavian temperatures during spring and autumn, when their abundance can be high [24]. Biting midges are able to move between different resting sites on a farm in order to optimize the conditions. The distance to which they are willing to move to find a suitable resting habitat is not known. However, Myers [25] found that biting midges can move up to 800 m in The Bahamas. Bidlingmayer [26] found *C. impunctatus* in Scotland, dispersed around 75 m from the original site of detection. Kirkeby et al. [27] recaptured marked biting midges at a distance of 1.75 km from their release point in Denmark.

The importance of microclimates has been emphasized in previous studies. For example, studies of highland malaria showed that anophelines may rest at warmer indoor temperatures [16, 28, 29], but were also found to be important in sea-level urban settings in Chennai, India where microclimatic temperature contributed to a shortened EIP for both vivax and falciparum malarial parasites [14]. Other studies have shown that air temperature has a substantial impact on malaria transmission across Africa [30]. Microclimatic temperatures provide significantly different estimates of vector-borne disease transmission parameters compared to meteorological temperatures [10]. Microclimatic temperatures allow for a faster pathogen development in biting midges (Schmallenberg and bluetongue virus) and mosquitoes (malaria, dengue, Dirofilaria and West Nile virus), more rapid digestion of blood meals, and a longer transmission season compared to meteorological temperatures [10].

Cattle farms may have one temperature recorded/modeled by the national meteorological service for any particular time period, but the farms are often surrounded by a number of microhabitats (vegetation) with potentially different microclimatic temperatures [10]. A previous study in Denmark showed that four microclimatic habitats located within a 1 km radius included a wide variation in temperature, and the temperature of these habitats was different from the nearest Danish Meteorological Institute (DMI) weather station [10]. Furthermore, the microclimatic temperature varied in different microhabitats, in different seasons and in different altitude. For example, the dry meadow was in general warmer than the hedges, wet meadow and forest. During spring, the lower heights of the dry meadow were warmer than the upper and mid-height whereas, during summer and autumn, the temperature at the lower habitats became cooler. The variation in vegetation types in different seasons played a vital role in changing the microclimatic temperature of different habitats [10]. Therefore, it is important to understand the microclimatic temperature of insect habitats surrounding the cattle farm over time and space. In this study, we explored the temperature of potential resting sites for *Culicoides* spp. from existing microclimatic habitats at all Danish cattle farms over 17 consecutive transmission seasons. The objective of this study was to quantify the variation in the EIP of Schmallenberg virus among these farms, and to identify possible spatial and temporal patterns of the EIP using the generated microclimatic temperatures.

Methods

We obtained geographical coordinates of each cattle farms from the Danish Central Husbandry Register (CHR) [31].

Estimation of microclimatic temperatures at Danish cattle farms

We obtained meteorological data from the implementation of the numerical weather prediction model system HIRLAM (High-Resolution Limited Area Model) at the Danish Meteorological Institute (DMI). Details of the dynamical and numerical aspects of the model can be found in the HIRLAM Scientific Documentation [32], and the DMI implementation is described by Sass et al. [33]. The meteorological data, dating back to the year 2000, are available in a circumpolar horizontal grid. The grid covers Europe and large parts of northern Asia and the Atlantic at a spatial resolution of approximately 15 km, and has an hourly time resolution. At the synoptic times 0, 6, 12 and 18 UTC (Coordinated Universal Time), the model assimilates a large number of the various different meteorological observations available in the geographical domain. The model calculates the initial state for the model integration. This analyzed state is a solution to governing equations of the atmosphere, as implemented in the model, in accordance with the observational data [26]. The model predictions at the intermediate synoptic hours (1–5 UTC, 7–12 UTC, and 19–24 UTC) are used.

In this study, we used the model temperature at a height of 2 m above the ground. We obtained the hourly meteorological temperatures, solar radiation, wind speed and humidity for each cattle farm according to the nearest model grid point. We then quantified the area of each of the different land covers within a radius of 500 m of each cattle farm in Denmark (n = 22,092) using the CORINE Land Cover database, 2006 [34]. We used CORINE Land Cover level 3 to classify the land cover, as it provided the highest resolution of vegetation information [34]. In total, 49 different types of land cover are described in the CORINE database, 25 of which we assumed to be suitable vector habitats. We did not have suitable microclimatic models for four of the 25 CORINE land covers (beaches, dunes, sands, bare rocks, and burnt areas). We reclassified the remaining 21 land covers into four major habitats: (i) dry meadow (non-irrigated arable land, rice field, pasture, permanent crops, complex cultivation patterns, natural vegetation, natural grasslands, moors and heathland, sparsely vegetated area); (ii) hedges (fruit trees and berry plantations, transitional woodland-shrub, vineyards, olive groves); (iii) wet meadow (permanently irrigated land, inland marshes, intertidal flats, estuaries); and (iv) forest (agro-forestry areas, broad-leaved forest, coniferous forests, mixed forest, sclerophyllous vegetation). These four microhabitats are described by Haider et al. [10]. We then regrouped the CORINE land cover surrounding the cattle farms into these four major microhabitat types, omitting land cover types that could not be reclassified

into these microhabitat types. We estimated the hourly microclimatic temperatures at three different heights, using recently published microclimatic temperature prediction models for dry meadow, wet meadow, hedges, and forest [10]. In this study, we considered the temperature at 0.55 m above ground for dry meadow, 2.2 m above ground for hedges, 6.8 m above ground for forest, and 0.50 m above ground for wet meadow, based on a literature review [15, 22, 35], expert opinion, and our assumption that these heights were representative of *Culicoides* spp. resting sites.

The microclimatic temperature prediction model

The microclimatic model uses hourly standard meteorological recordings as input variables to predict the hourly microclimatic temperature of a particular habitat [10].

Microclimatic temperature = meteorological temperature + meteorological temperature the previous hour + solar radiation + wind speed + humidity + month weight (from May to October) + time of day + (solar radiation * wind speed) + (solar radiation * month) + (wind speed * height above ground) + (solar radiation * height above ground)

The weight of months was calculated with the formula

$$\text{Month (x)} = 15 - (\text{absolute (date)}/\text{No.of days of month})$$

The fitted model was used to predict hourly microclimatic temperature for each of the Danish cattle farm for the period of 2000–2016.

The microclimatic models were developed for the period May to October [10]. We furthermore estimated the microclimatic temperature for the period April to November with the assumption that the habitats would remain the same in November as for October, and April would be the same as for May.

Since the precise resting sites of *Culicoides* spp. are not known, we assumed that vectors would select a resting habitat randomly and that this would be proportional to the availability of the habitats around the farms. If for example, a farm was surrounded by 60% dry meadow and 40% forest, we assumed the 60% of the vectors would permanently rest in the dry meadow and 40% would permanently rest in the forest. However, vectors may actively select a resting habitat according to a preferred temperature or other criteria. To quantify the potential for disease transmission in case vectors actively select a preferred habitat every hour, we estimated the EIP at both the warmest and the coldest habitats available at each farm. We compared the EIP estimated by the different microclimatic temperatures to the EIP estimated by the DMI-modeled temperature for the nearest grid point to each cattle farm.

Estimating the EIP of Schmallenberg virus

We estimated the EIP of Schmallenberg virus using the following equation, originally developed for bluetongue virus serotype 9, but widely used for Schmallenberg virus [7]:

$$1/(0.019 \times (\text{T}-13.3)) \text{ where T is the hourly temperature } (^{\circ}C)$$

We developed a rate summation model; in which virus development was calculated hourly and summed up daily until virus development was complete (i.e. reached a value of 1). We used the estimated microclimatic temperature of all four classes of land cover (dry meadow, wet meadow, forest, and hedges) to estimate four different EIP for each farm. Assuming the vectors select resting sites completely randomly, we estimated a weighted average EIP (EIP_{rand}) for each farm based on the proportion of the four habitats surrounding each farm. We considered the maximum lifespan of *Culicoides* spp. to be 60 days, and the EIPs are presented as average values of the different microclimates at each farm and as averages of different farms. In the model, an EIP of 60 days on May 1st indicates that virus development would be completed on June 29th (60 days later) for any biting midges that ingested an infected blood meal with Schmallenberg virus on that day. Therefore, we included the temperature data up to November 30th so that we could allow 60 days after the last date of our EIP calculation (September 30th). When the temperatures at one or more habitats at a farm were too low for the EIP to complete in 60 days, it became problematic to calculate an average EIP for that farm. Realistically, these sites did not have a value for EIP, as values greater than the lifespan of *Culicoides* spp. are not plausible. However, omitting these cooler sites from the average EIP of a farm would artificially shorter the average EIP by selectively removing the coolest microclimates from the average. To be able to present average estimates of EIP for a farm, we allocated a value of 61 days to the EIP of habitats where virus development was not possible in 60 days. But when the average EIP of all habitats on a farm reached a value of over 60 days, we concluded that EIP could not be completed at that farm.

Vectors may not select their resting sites randomly but may instead be able to move and select a favorable microclimate every hour. We therefore identified the maximum and minimum hourly temperature among the four habitats surrounding each farm and used these time series to estimate the maximum temperature EIP (EIP_{maxT}) and the minimum temperature EIP (EIP_{minT}). We also estimated the EIP using DMI's modeled temperature (EIP_{DMI}). We estimated the EIP for each transmission period (April 1st - September 30th) for the years 2000–2016, and finally calculated a 17-year average EIP for each season:

spring (April 1st - May 31st), summer (June 1st - August 31st) and autumn (September 1st - September 30th). Finally, to supplement the modeled microclimatic temperatures, we also calculated the EIP using hourly maximum and minimum microclimatic temperature recorded in the field at Strødam, 30 km North of Copenhagen, Denmark, during 2015. The details of the data collection are described in Haider et al. [10]. The EIP model was developed using the statistical software SAS [36]. We used the statistical software R version 3.4.0 (packages "*raster*", "*maptools*" , "*rgdal*", "*plyr*", "*foreign*" "*lubridate*") to predict the hourly microclimatic temperature for each year in order to perform summary statistics of temperature and EIP data and to produce all figures [37]. All maps were prepared in the geographical software QGIS [38].

Results

Land cover

There were 22,092 cattle farms in the CHR database. Of these, 22,004 farms were surrounded by at least one of the four habitats: dry meadow (83% of farm areas), hedges (6%), wet meadow (3%) and forest (3%). The remaining 5% of farm areas were covered by habitats for which we had no model to estimate the microclimatic temperature (e.g. beaches, dunes, sands, etc.). The remaining 88 farms (0.4%) either did not contain any habitats included in the microclimate model, or contained habitats that were not suitable as vector-resting sites. We excluded these farms from further analysis. Of the 22,004 farms, 8448 (38%) had only one of the four types of land cover: 8444 had only dry meadow, three had only hedges and one farm had only forest within a 500 m radius.

Comparison of microclimatic and DMI-modeled temperatures of Danish cattle farms

The average daily minimum, maximum and mean temperature of each of the four habitats surrounding the cattle farms for the 17 year-period are summarized in Fig. 1, together with the standard DMI temperature. The estimated microclimatic temperatures differed considerably from the DMI temperature. This difference was larger in spring and autumn than summer. In spring, the daily maximum temperature varied (5th and 95th percentiles) from 9.0 °C to 17.9 °C (DMI-modeled), 13.7 °C to 26.2 °C (dry meadow), 13.9 °C to 24.1 °C (hedges), 12.1 °C to 20.6 °C (wet meadow), and 10.7 °C to 18.9 °C (forest). During the same period, the minimum temperature varied (5th and 95th percentiles)

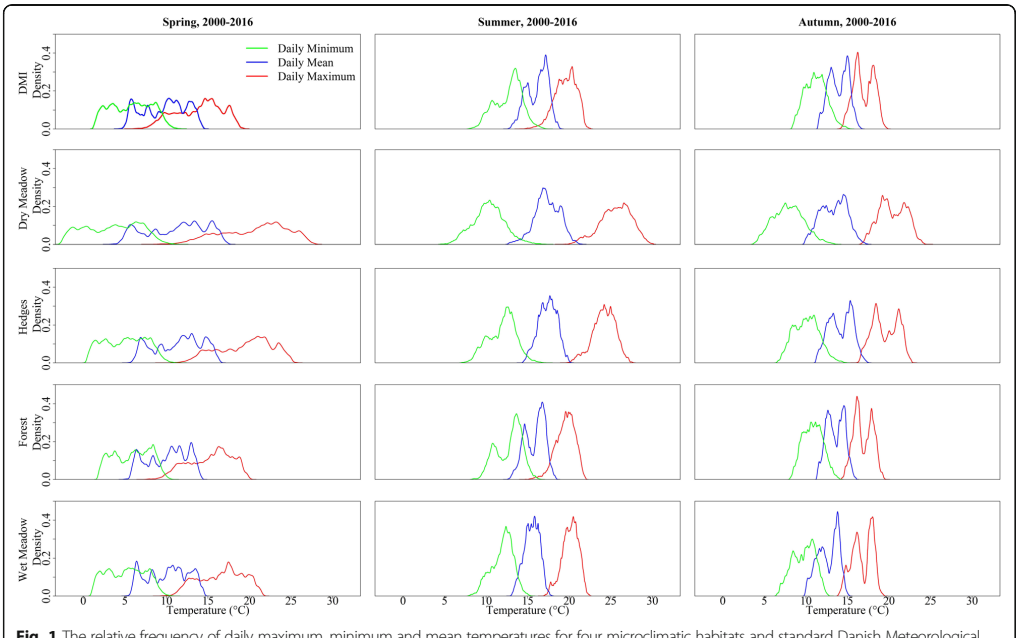

Fig. 1 The relative frequency of daily maximum, minimum and mean temperatures for four microclimatic habitats and standard Danish Meteorological Institute (DMI) temperatures surrounding Danish cattle farms (500 m radius for microclimatic habitats), based on hourly temperature data. The figure represents 17-year (2000–2016) averages for spring (April-May), summer (June-August), and autumn (September)

from 2.0 °C to 9.5 °C (DMI-modeled), -1.7 °C to 8.4 °C (dry meadow), 1.1 °C to 8.9 °C (hedges), 1.6 °C to 8.7 °C (wet meadow), and 2.4 °C to 9.1 °C (forest). The dry meadow habitats had the most extreme temperatures, with the warmest daytime temperatures and coldest nighttime temperatures during the period April to September 2000–2016 (Fig. 1). On average, the daily maximum temperature in dry meadow, hedges, wet meadow, and forest was 3.9 °C, 3.1 °C, 0.9 °C and 0.4 °C higher than the DMI daily maximum temperature, respectively. The average daily minimum temperature in dry meadow, hedges, wet meadow, and forest was 3.4 °C, 1.1 °C, 1.1 °C and 0.1 °C lower than the DMI daily minimum temperature, respectively. The DMI estimates for daily maximum temperature of different farms located in different parts of the country varied (5th and 95th percentiles) from 10.4 °C to 21.1 °C (difference: 10.7 °C), and the daily minimum temperature varied (5th and 95th percentiles) from 1.0 °C to 14.3 °C (difference: 13.3 °C). The warmest habitat of a farm had an average temperature that was 3.7 °C (5th and 95th percentiles: 1.0–7.8 °C) higher than the coolest habitats of the same farm.

Spatial variation of temperature in Denmark

To quantify how temperature varied spatially on a particular day, we plotted the minimum and maximum temperature for each farm on May 1st, July 1st and September 1st in four selected years: 2000, 2005, 2010 and 2016 (Fig. 2). This showed a wide variation in daily temperatures in Denmark. For example, on May 1st 2016, the maximum temperature varied (5th and 95th percentiles) from 15.5 °C to 22.2 °C in dry meadow, 16.4 °C to 20.3 °C in hedges, 12.4 °C to 15.5 °C in wet meadow, and 11.1 °C to 15.2 °C in forest habitats, compared to a variation of 9.7 °C to 13.5 °C in DMI-modeled temperatures (Fig. 2). The minimum temperature on the same day varied (5th and 95th percentiles) from -2.9 °C to 3.2 °C in dry meadow, 0.1 °C to 4.8 °C in hedges, 0.6 °C to 4.6 °C in wet meadow, and 1.6 °C to 5.6 °C in forest

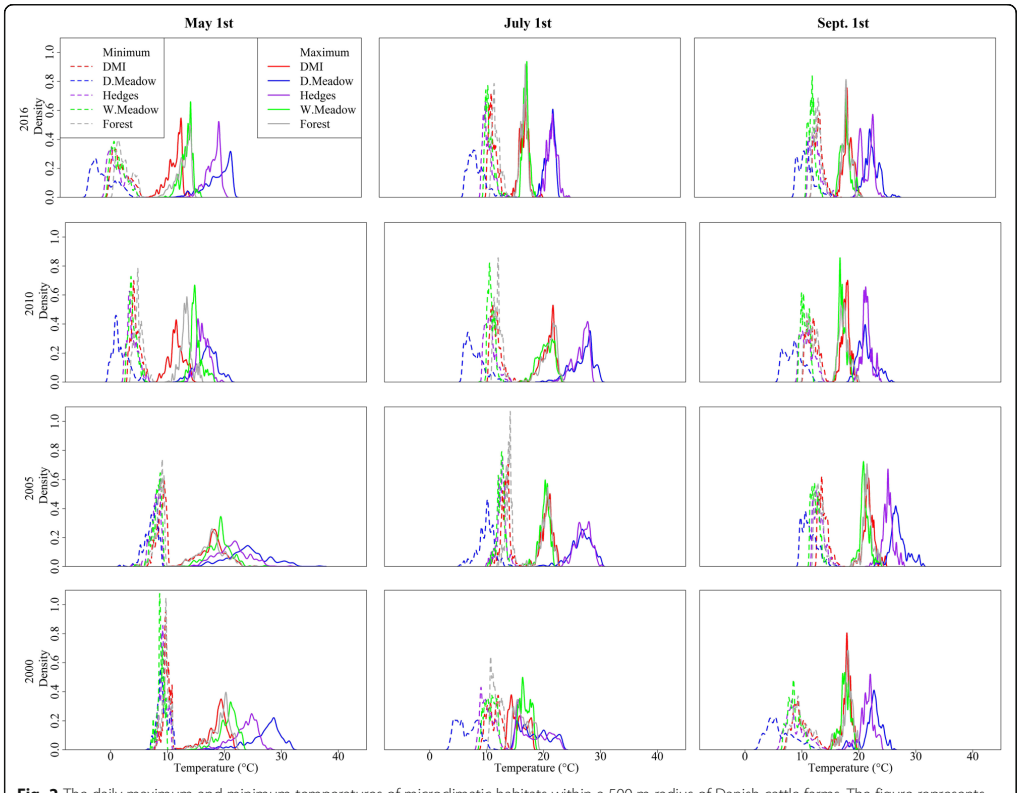

Fig. 2 The daily maximum and minimum temperatures of microclimatic habitats within a 500 m radius of Danish cattle farms. The figure represents the daily minimum and maximum temperatures on May 1st, July 1st and September 1st for four selected years: 2000, 2005, 2010 and 2016

habitats, compared to 0.8 °C to 5.4 °C in DMI-modeled temperatures (Fig. 2).

Comparison of the EIP of Schmallenberg virus estimated from different temperatures in Danish cattle farms

The mean EIP of Schmallenberg virus (5th and 95th percentiles) for all cattle farms during spring, summer, and autumn for the 17-year period was: 23 (18–33), 14 (12–18) and 51 (48–55) days, respectively, assuming that vectors select resting sites randomly. These estimated EIP values were much shorter than the EIP generated from DMI temperatures, which were: 43 (39–52), 21 (17–24), and 57 (55–58) days, respectively. The EIP of Schmallenberg virus estimated from random resting sites was comparable to the EIP estimated from the hourly maximum temperatures at the farms for the same three periods: 20 (17–26) days (spring), 11 (10–13) days (summer), and 46 (42–50) days (autumn). However, the EIP estimated when vectors were assumed to select the minimum hourly temperatures at the farms were much longer: 44 (39–53) days in spring, 30 (26–36) days in summer, and 59 (59–60) days in autumn (Fig. 3).

Annual variation in Schmallenberg virus EIP

There was a large year-to-year variation in the EIP of Schmallenberg virus over the three seasons for the period 2000–2016 (Fig. 4).The mean EIP of Schmallenberg virus in the spring of two consecutive years, 2015 and 2016, was 31 and 19 days using random resting-site temperatures, 27 and 18 days using hourly maximum temperatures, 56 and 34 days using minimum hourly temperatures, and 55 and 33 days using DMI temperatures (Fig. 4). In general, the EIP of Schmallenberg virus infections starting in the vectors during summer was shortest, followed by infections starting in spring and autumn (Fig. 4). In the spring of 2012, Schmallenberg virus was detected in a malformed calf born in Denmark, when the mean EIP for all Danish cattle farms was 24 and 42 days based on an estimation of random resting-site and DMI temperatures, respectively. The cow was probably infected during the autumn of 2011, when the mean EIP was 48 and 59 days, respectively.

Spatial variation in EIP

The variation in EIP over a short time period (e.g. 1 day) differs due to the spatial variation of temperatures in farms located in different parts of the country. We

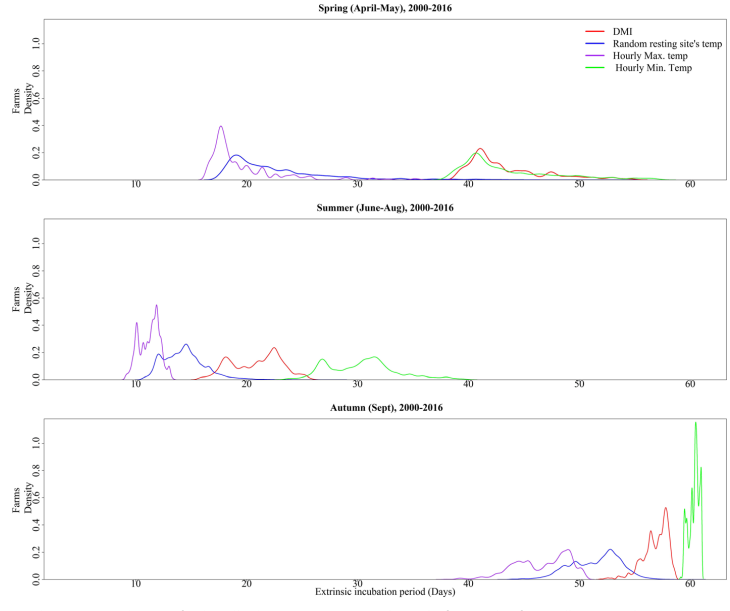

Fig. 3 The extrinsic incubation period (EIP) of Schmallenberg virus on Danish cattle farms. The figure illustrates the 17-year average EIP for spring (April 1st - May 31st), summer (June 1st - August 31st), and autumn (September 1st - September 30th), assuming that vectors either randomly select a resting site according to the area this habitat occupies within a 500 m radius of the farms (random resting-site), or the warmest (hourly maximum temperature) or coldest (hourly minimum temperature) available farm habitat each hour, or that they rest at the nearest DMI temperature grid point (DMI)

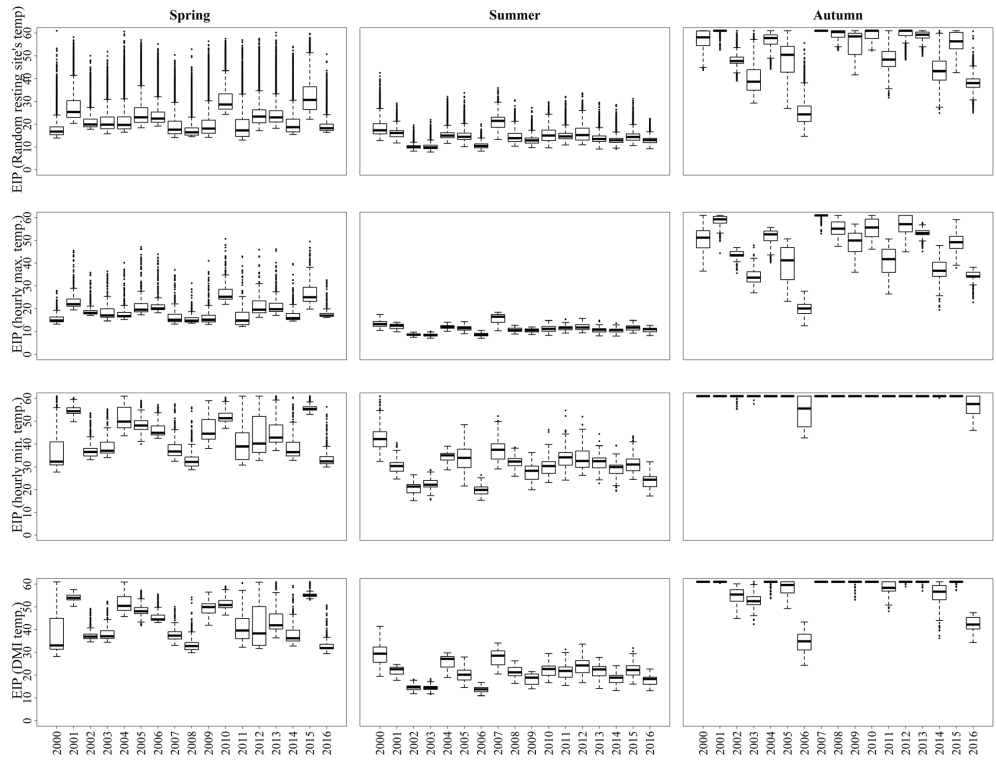

Fig. 4 The annual variation in the extrinsic incubation period (EIP) of Schmallenberg virus between 2000 and 2016 at Danish cattle farms in: spring (April-May), summer (June-August), and autumn (September), assuming that vectors randomly select a resting site according to the area this habitat occupies within a 500 m radius of the farms (random resting), or that the vector chooses the warmest (maximum temperature) or the coldest (minimum temperature) available farm habitat every hour or that they rest at the nearest Danish Meteorological Institute (DMI) temperature grid point. The bottom and top of the box indicate the first and third quartiles; the band inside the box is the median. The dots outside the box are outliers

plotted the distribution of EIP_{rand} estimated based on random resting sites' temperature for specific dates (May 1st, July 1st, and September 1st) for each of the 17-year period for all Danish cattle farms in order to examine the geographical variation in Schmallenberg virus transmission potential (Fig. 5). There was a large variation in EIP (5th and 95th percentiles) between farms: 9–19 days on May 1st, 2000, 21–40 days on May 1st, 2005, 23–43 days on May 1st, 2010, 25–56 days on May 1st, 2015, and 10–21 days on May 1st 2016 when modeled with temperatures from random resting sites. For July 1st, the estimates (5th and 95th percentiles of EIP) were: 16–23 days in 2000, 9–11 days in 2005, 6–11 days in 2010, 6–15 days in 2015, and 12–20 days in 2016. For September 1st, the estimates were: 29–60 days in 2000, 11–32 days in 2005, 24–60 days in 2010, 18–60 days in 2015, and 11–16 days in 2016 (Fig. 6). The daily variation in EIP between farms was larger in May and September. A large geographical variation in EIP on a particular day was observed over the 17-year period (Fig. 5).

Geographical patterns of Schmallenberg virus EIP in Denmark

In general, cattle farms located in the southeastern part of the country (comprising southern Funen and associated islands, Lolland, Falster, and southern Zealand) had a shorter EIP. Farms located in Jutland, especially those in the north-west (comprising Thisted and Herning), had a longer EIP (Fig. 6). This pattern applied to all calculations of the EIP, whether we assumed that vectors were resting at random resting site temperatures, at maximum temperatures, at minimum temperatures or at the DMI temperatures. The maps based on the selection of random resting sites showed that farms with a shorter

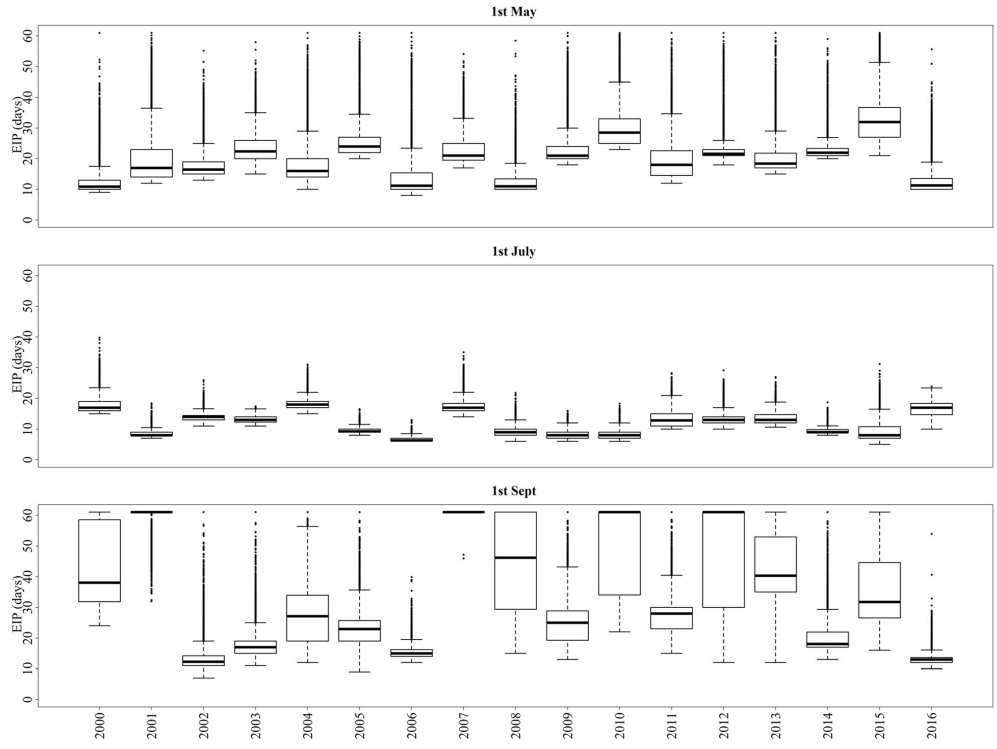

Fig. 5 The virus development time (extrinsic incubation period) in *Culicoides* spp. vectors when infected with Schmallenberg virus on Danish cattle farms on May 1st, July 1st and September 1st during the period 2000–2016, assuming that vectors randomly select a resting microclimatic site according to the area this habitat occupies within a 500 m radius of the farms. The bottom and top of the box indicate the first and third quantiles, the band inside the box is the median. The dots outside the box are individual outliers

EIP (in red) were surrounded by a number of farms with a longer EIP (in blue). This indicates that land cover around the farm plays an important role in determining the EIP of Schmallenberg virus, rather than a climatic geographical trend alone. At minimum hourly microclimatic temperatures, the EIP could not be completed in over half of the farms (n = 12,030, 54.7%) during the autumn.

Variation in EIP with different types of temperature

On average, from April 1st to September 30th, the minimum number of days required for completion of the EIP was: 10 days with the random resting-site temperature, 9 days with the hourly maximum temperature, 20 days with the hourly minimum temperature, and 14 days with the DMI temperature (Fig. 7). The range of the EIP for the year 2015 at Strødam was 4–23 days based on the observed maximum microclimatic temperature and 19–60 days based on the observed minimum microclimatic temperature (Fig. 7). When assuming that the vectors selected the lowest available temperature at each farm for each hour for the entire transmission season (April 1st to September 30th), the EIP was on average 2.3 (range: 1.1–4.1) times longer than the EIP for the same farm with vectors assumed to select the maximum microclimatic temperature. The EIP based on random resting-site temperatures was shorter throughout the transmission seasons than the EIP estimates based on DMI temperatures. The EIP based on random resting-site temperatures also showed a longer season of transmission.

Land cover and EIP (based on random resting sites' temperature)

The mean EIP for farms with 10% dry meadow varied (5th and 95th percentiles) from 25–33 days, whereas the estimates varied from 21–29 days for the farms with 75% dry meadow. The mean EIP for farms with 10% forest varied (5th and 95th percentiles) from 23–30 days,

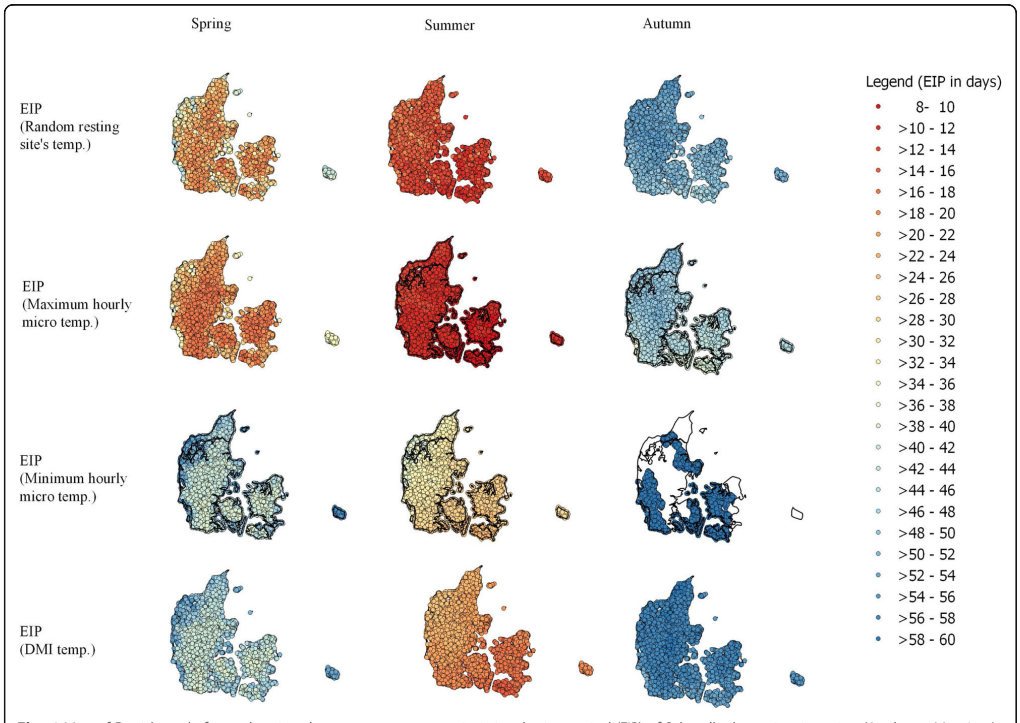

Fig. 6 Map of Danish cattle farms showing the 17-year average extrinsic incubation period (EIP) of Schmallenberg virus in spring (April 1st - May 31st), summer (June 1st - August 31st), and autumn (September 1st - September 30th) using random resting-site temperatures, Danish Meteorological Institute-modeled (DMI-modeled) temperatures, and maximum and minimum hourly microclimatic temperatures. The EIP is generated from our virus development model using estimated hourly microclimatic temperatures. The white color indicates the farms where virus development is not possible in 60 days on average

whereas the estimates varied from 27–35 days for the farms with 75% forest. This suggests that the proportion of warm and cold land cover around the farm had an important role in driving farm-level EIP.

Discussion

Sixty-three percent of infectious diseases in Europe are climate sensitive and 82% of these are sensitive to temperature alone and vector-borne diseases have been identified as the most temperature-sensitive diseases [39]. Our study generated one of the largest microclimatic temperature datasets in Europe. The dataset is useful for modeling temperature-sensitive diseases of livestock and diseases with zoonotic potential as microclimatic temperatures of Danish cattle farms are different from the temperatures modeled by National Meteorological Institute, DMI. While a single temperature is modeled by DMI for a specific geographical location, we found that approximately 62% farms had more than one type of land cover and therefore more than one microclimatic temperature at a specific point in time. Dry meadow was the most abundant habitat type and had the warmest microclimatic temperature, whereas wet meadow was the coolest and least abundant habitat type in Denmark.

The microclimatic habitats surrounding the farms were 0.4 °C to 3.9 °C warmer or 0.1 °C to 3.4 °C cooler than the DMI-modeled temperature. Daily temperatures observed on farms located in different parts of the country could vary by a maximum of 10.7 °C to 13.3 °C based on standard meteorological office data, but there were microhabitats within a 500 m radius on a farm in which temperatures could vary by a magnitude of 1.0 °C to 7.8 °C (mean: 3.7 °C) each hour. This emphasizes the importance of variation in microclimatic habitat temperature and the need to incorporate it into vector-borne disease-transmission models. Similar conclusions were made from a microclimatic study in Georgia, USA, where researchers concluded that the climatic condition captured by local weather station data did not reflect the microclimatic temperature experienced by

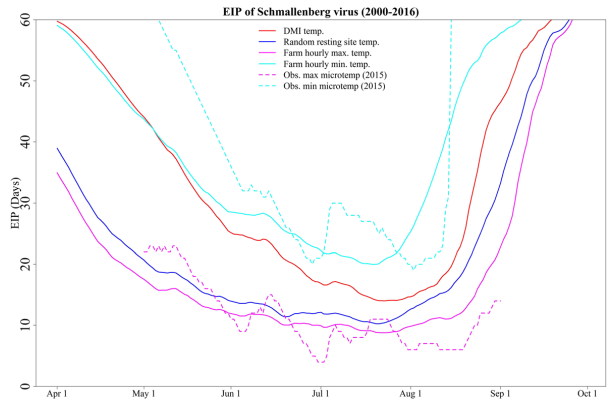

Fig. 7 The daily average extrinsic incubation period (EIP) of all cattle farms for the period 2000–2016 (April 1st - September 30th). The estimated EIP using random resting-site temperature (blue line) showed a shorter virus development period compared to the Danish Meteorological Institute (DMI) temperature (red line). Using estimated maximum and minimum hourly temperatures at Danish cattle farms generated two extreme scenarios of EIP (purple and cyan lines). The observed microclimatic temperature from 2015 only showed real annual variation in EIP for Schmallenberg virus for this specific year (dotted line). The observed field data from Strødam were available from May 1st, 2015 to September 1st, 2015

the mosquitoes [13]. Another study conducted in rural Argentina showed that microhabitats were generally 5.0–5.6 °C warmer than the ambient temperature [40]. A further study in tropical urban settings in Chennai, India reported higher daily mean temperatures in microhabitats than was recorded by weather stations [14]. A study conducted in the Netherlands showed similar daily mean temperature recorded from national meteorological institute and microclimatic data loggers but the daily temperature variation was much larger in microclimatic habitats [15]. Such variation might have a large impact on virus development and insect survival [15, 41]. The differences in temperature modeled by DMI and the temperature, we predicted for microclimatic habitats, resulted in a large variation in the estimates of EIP which is in an agreement with the Chennai, India study [14]. Here the EIP for both the vivax and falciparum malarial parasites was found to be 1–4 days shorter using measured microclimatic temperature compared to meteorological temperatures [14]

An important finding of this study was the large between-farm variation in the EIP of Schmallenberg virus. Denmark is a small country of 42,931 km^2, throughout which the mean monthly temperature does not vary more than 2 °C. It has therefore been assumed that vector-borne diseases have only small climate-driven variations in transmission patterns. However, we found a large variation in the EIP for cattle farms located in different parts of the country. The EIP of Schmallenberg virus varies from the coolest to the warmest site on a farm by a factor of 1.1 to 4.1. This means that the virus could develop in the biting midges in seven days in one type of habitat at a cattle farm, and up to 29 days in another habitat at the same farm, despite the midges being infected on the same day.

We found a consistent geographical pattern that showed farms with shorter EIP for Schmallenberg virus were grouped together in the southern parts of the country. Such microclimatic hotspots are important as they may help veterinary authorities prioritize areas for surveillance and allocate resources to prevent and control potential outbreaks, e.g. by increasing vaccination cover locally. This finding has practical implications for Denmark and similar areas in temperate climates around the world. Countries and territories may need to implement strategies to identify, control and prevent vector-borne diseases based on how rapidly a virus can develop within the area, and farm habitats might play a vital role in such decisions. Particular attention may be necessary for parts of a country that is rich in a particular habitat thought to increase the risk of vector-borne disease transmission (e.g. dry meadow). While performing risk assessments for vector-borne diseases, the farm-level potential for disease transmission (e.g. EIP) should be assessed thoroughly, together with other important transmission parameters that can vary spatially, e.g. vector abundance and host densities.

There is an increased concern that climate change might affect the transmission of vector-borne disease in terms of greater geographical expansion of existing diseases and a higher number of outbreaks in endemic areas[42] as climate change may increase the reproductive rate of the insect, the insect biting rates, and shorten

the pathogen incubation period [43]. The Intergovernmental Panel on Climate Change (IPCC) have projected a rise in temperature of *c*.0.2 °C per decade over the next two decades [44]. In our study, we found that *Culicoides* spp. have available microhabitats surrounding a farm that are on average 3.7 °C warmer than the coolest habitats. If the biting midges can choose habitats optimally, the variation in the microclimatic temperatures they can be exposed to is much larger than what is predicted due to global warming over the next two decades. It has been suggested that man-made changes in highland Africa have caused an increase in microclimatic temperatures, thereby increasing the vector abundance and facilitating malaria transmission [45]. Climate change could worsen the condition in future [42, 43], but the impact can potentially be counteracted by a change in vector-resting behavior, or by a change in land cover. This has been shown in studies in Austria, where land cover classes were reported to be the most significant factor for the abundance and distribution of mosquitoes [46]. In Uganda, replacement of natural swamp vegetation with agricultural crops led to increasing temperatures, contributing to higher malaria transmission [45]. A change in resting sites could lower the resting temperature of vectors even if the global temperature increases. Such a change may have a bigger impact than years of global warming. Mordecai et al. [47] showed that a 6 °C temperature decrease in the optimum temperature for malarial pathogen development is equivalent to a century of temperature change projected by worse-case climate change scenarios. Therefore, there may not be a simple relationship between global warming or increasing temperatures and vector-borne disease transmission. Instead, the impact is complicated and highly dependent on the microhabitat of the resting sites as well as the vector-resting behavior as shown in earlier studies of malaria in East Africa [41]. Here they evaluated the malaria parasite development rate at different temperatures and found that mosquitoes resting indoors at warmer temperatures could transmit malaria between 0.3 and 22.5 days earlier than mosquitoes resting at colder outdoor temperatures [41].

We found a wide range of EIP for three different estimates of microclimatic temperatures and the standard DMI temperatures. For example, the minimum number of days required to complete the EIP using the warmest hourly temperature for a farm over the entire transmission season (April-September) was 9 days, whereas the estimate was 20 days for the coolest hourly microclimatic temperature. Therefore, the choice of input temperature has a very large impact on the model outcome, stressing the importance of selecting appropriate temperatures for modeling vector-borne diseases. Standard meteorological temperatures are often used for modeling vector-borne disease, yet it is not reasonable to assume that these will represent an average of the actual resting-site temperatures.

Although we do not know the precise location of vector-resting sites, a number of studies have looked at the resting sites of biting midges [15, 20–22, 25, 26]. These studies showed that biting midges can choose habitats between a few centimeters and 10 m above ground, and can choose favorable microclimatic habitats from up to 1.75 km [15, 21, 27]. It has recently been shown that land cover type could significantly affect the distribution of mosquitoes [46]. They might select shaded and humid places during the warm hours of the day, and warmer areas during cooler periods of the day/night. Humidity, shade, and temperature may all play an important role in resting-site selection, but the temperature will ultimately affect virus development. This again emphasizes the need for a better understanding of insects' selection of resting sites, to identify the appropriate temperatures for modeling vector-borne diseases.

Our estimates of EIP support the empirical findings in Denmark and other European countries [14, 17, 48]. On September 30th, the mean EIP of Schmallenberg virus was 21 days using hourly maximum temperatures, 35 days using random resting-site temperatures, 49 days using DMI temperatures and 60 days using hourly minimum temperatures. This shows that even at minimum microclimatic temperatures, biting midges will be able to transmit the virus 60 days later (i.e. in the last week of November). Pregnant ewes and cows infected in mid to late November will give birth to malformed lambs/calves around March-April and August-September the following year. Schmallenberg virus has recently been identified in aborted sheep/cattle during spring in Belgium [48] and in Denmark [4]. We found a relatively long season of transmission when modeled with microclimatic temperature compared to that of DMI temperature. We considered the maximum lifespan of biting midges to be 60 days. In reality, the survival of biting midges depends on many factors and the lifespan of *Culicoides* spp. has been documented to vary widely, from 10 to 90 days [49]. We estimated the EIP of Schmallenberg virus for the period of April 1st to September 30th, deeming this to be warm enough to facilitate vector-borne disease transmission.

In an extreme scenario, we found four days to be the minimum required time to complete the EIP using the observed (maximum) microclimatic temperature recorded at Strødam, Denmark. This indicates that virus development could take just over half a week, even in Scandinavian climates.

The EIP estimated using random resting-site temperatures was very similar to the EIP estimated using the

hourly maximum temperature at a farm. This is because the dry meadow was the dominant microclimate (83%) in Danish cattle farms, and this type of habitat being the warmest microclimate among the four habitats included in this study. Therefore, the average farm-level EIP of random resting sites was highly influenced by the dry meadow temperature. The EIP estimated from DMI was consistently longer than the estimates derived from the hourly maximum temperature at a farm, and even the estimates derived from the temperature of random resting sites. Thus, modeling with DMI temperatures will lead to an underestimation of the real potential of vector-borne diseases.

Conclusions

We estimated a wide range of the EIP of Schmallenberg virus from different microclimatic and DMI temperatures, which highlights the importance of selecting appropriate temperatures for modeling vector-borne diseases. At any given time, the EIP could vary more than fourfold between the coolest and the warmest microclimates of a cattle farm. This finding has important implications for Denmark and other temperate areas around the world, as countries may need to implement strategies for the control and prevention of vector-borne diseases based on the potential for transmission in different geographical areas. The between-farm variation in EIP is large, with a geographical trend suggesting that disease transmission may vary substantially among regions, even in a small country like Denmark. This could be useful when designing risk-based surveillance for emerging and re-emerging vector-borne diseases. To maximize the use of the available resources, surveillance may focus on geographical areas most at risk (for example, farms surrounded by dry meadow) and on high-risk periods (for example July and August), while also taking into consideration other important factors including vector abundance and host densities. About two thirds of cattle farms (62%) in Denmark had more than one type of land cover and therefore more than one microclimatic temperature. We have shown that warmer microhabitats available to *Culicoides* spp. around farms had on average 3.7 °C higher temperatures compared to the cooler available habitats of the same farm. Man-made changes to the habitats surrounding the farms could alter the risk of vector-borne disease transmission in the future. The completion of virus development (and thereby the transmission potential for vector-borne diseases) will be determined by the temperatures of the actual microclimatic habitats in which the vectors rest. This emphasizes the need for better knowledge on the behavior behind insect resting-site selection to enable selection of appropriate temperatures for modeling vector-borne disease transmission. The farm-level microclimatic hourly temperature dataset generated in this study is one of the largest (22,004 farms, each for 8 months for 17 years) used for studying infectious and vector-borne diseases driven by temperature. In the absence of known resting sites, we recommend using the range of possible microclimatic temperatures available.

Abbreviations

CHR: Central husbandry register; CORINE: Coordination of information on the environment; DMI: Danish Meteorological Institute; EIP: Extrinsic incubation period; HIRLAM: High-resolution limited area model; QGIS: Quantum global information system; SAS: Statistical analysis software; UTC: Coordinated universal time

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Availability of data and materials

All the summary data are disclosed in the text, figures or maps of the article. The raw datasets of microclimatic temperature that were used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

NH led the data analysis and manuscript writing, ACC helped extract CORINE Land Cover and prepare maps, LJK helped to interpret the data analysis and provided critical input in manuscript writing, JHS provided the meteorological data and helped to analyze and interpret the microclimatic and DMI data, RB planned the original study, helped with data analysis, developed the EIP model and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Manuscript III

Quantifying the potential for bluetongue virus transmission on Danish cattle farms

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Manuscript in preparation

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Abstract

We used a mechanistic transmission model to estimate the daily number of infectious bites (IB) with bluetongue virus (BTV) using estimated microclimatic temperatures at 22,004 Danish cattle farms for the period 2000-2016, and *Culicoides* midge abundance based on 1,453 light trap collections in 2007-2016. We used published information on relationships between temperature and four key transmission parameters: extrinsic incubation period (EIP), daily vector survival rate, daily vector biting rate and the rate of BTV transmission from host to vector. We quantified the uncertainty and sensitivity for each parameter associated with daily IB estimates.

We estimated a maximum daily IB of 2,899, while the best-case scenario suggested transmission was not possible. The mean (10-90th percentiles) daily IB was 2.2 (0-8.9) during the transmission period. Variation in the vector survival and BTV host-to-vector transmission rates resulted in large uncertainty in the predictions. Several peaks of vector abundance were observed, and peaks in the vector population and IB coincided. Temperature and vector abundance were the most influential parameters in the estimates of daily transmission potential.

Our model predicted a potential for BTV transmission in Denmark, with a high uncertainty in the estimation of daily IB with BTV.

Introduction

Bluetongue virus (BTV) causes bluetongue disease (BT) – one of the most important diseases of ruminants, which is notifiable to the World Organization of Animal Health (OIE) [1,2]. Symptoms of BT include weight loss, abortion, reduced milk yield and ultimately death in ruminants. BT is responsible for international trade restrictions on animals and animal products [1,2]. BTV causes global losses of an estimated 2.6 billion EUR a year [2]. Five different strains of BTV have been reported in Europe since 2006 [3], and these viruses in combination have caused the most severe outbreaks of BT ever reported, resulting in the death of over 1.5 million sheep [1,4]. In Denmark, BTV was first identified in 2007, and another 15 outbreaks were identified across the country in the 2008 [5].

Estimating the vectorial capacity (VC) of a vector-borne disease (VBD) is a common approach used to evaluate the potential threat of disease outbreak once the disease has been introduced. The VC of BTV is defined as the number of new hosts infected every day from one infectious host. Values for VC can aid in estimating potential outbreak risks, the number of animals infected, and the time when local spread could occur. VC can be used as a risk assessment tool, where higher VC values indicate a faster spread [6]. The VC can be summed up over the infectious period (period of viremia) of the host to obtain the basic reproduction period of a disease. This reproduction period is commonly known as R_0 [7], and is defined as the average number of infected individuals originating from the introduction of one infected host into a naive population [1,6].

The VC of a vector-borne disease can be expressed as a function of the extrinsic incubation period (EIP – the time interval between ingestion of an infected blood meal and the ability to transmit the virus), the number of vectors per host, vector biting rate, and vector survival rate. A simple version of the VC, adapted from Garrett-Jones [8] and originally developed from Ross-Macdonald's R_0 model for Malaria [9] is shown as:

$$C = m * a^2 * p^n * (\frac{1}{-lnp})$$

where C is the VC, m is the number of vectors per host, a is the daily vector biting rate, p is the daily vector survival rate, and n is the duration (in days) of the EIP of the virus in the vectors. The EIP [10], vector biting rate, and vector survival rate are all highly dependent on temperature [11]. The EIP has a very strong impact on the VC of a disease, as the VC is proportional to the survival rate raised to the power of EIP. Other parameters that influence the VC include the transmission rate from host to vector and the transmission rate from vector to host. Recently, a temperature-dependent equation for BTV transmission from host to vector was suggested [12,13].

When calculating the VC, it is important to use the vector lifecycle traits accurately, including the lifespan of the vector, the total number of bites during the lifespan, and how many of these bites are infectious. The original and modified versions of the Ross-Macdonald-Jones equation assume a "fixed rate" for most of these parameters [14], whereas simulation models (e.g. SIR or SEIR model) use a range of values [13]. For VBD, many of the temperature-dependent parameters are correlated. For example, the EIP will be shorter at higher temperatures but so will the survival rate, while the biting frequency will be higher. Using these parameters independently in the VBD models may not provide a realistic transmission potential.

The EIP equation used for BTV modelling has two important aspects: i) there is a threshold temperature below which no development is possible [1,12,15–17], ii) above this threshold, there is either a linear or a more complicated non-linear relationship between the temperature and virus development [18]. The minimum threshold temperatures for virus development are often derived from a series of experiments based on constant temperatures [15–17,19], after which a model is fitted to the data. In reality the threshold temperature might be much lower [18], and temperatures climbing beyond this threshold will result in quicker virus development, followed by a period of more steady development. These widely used equations in R_0 modelling are likely to be less reliable at extreme temperature ranges, especially at the threshold

temperatures. Furthermore, using published equations, some strains of BTV develop at a higher rate immediately after the threshold temperature is exceeded, while some strains of BTV develop at a growing rate at increasingly higher temperatures.

Equations for the daily survival rate of *Culicoides* have mostly been developed from natural capture-release studies on insects [17,20]. Some of these equations differ substantially in their daily survival rates, for example Wittmann et al. [21] suggested a high daily survival, while Gerry & Mullens [17] suggested a low daily survival rate. Furthermore, many researchers assume the daily survival rate is independent of age [20], while others believe it will vary [22].

Virus transmission rates from host to vector and vector to host are two vital parameters that determine the VC of *Culicoides* [23,24]. A study by Paweska et al. (2002) used temperature-dependent transmission rates of BTV from host to vector for *C. imicola* and *C. bolitinos* [12]. However, there was a large variation in the estimated rates between the species. The maximum estimated transmission rate for *C. imicola* was 0.06 – less than one tenth that of *C. bolitionos* (0.64). These estimates will result in very different transmission rates.

Many of the models used in estimating vector-borne disease transmission are based on monthly or daily mean temperatures [11,16,25]. In reality, insects do not experience a "mean temperature", but are instead exposed to changing temperatures throughout the day [26]. Therefore, using hourly temperature can capture the real impact on VBD while modelling with temperature-sensitive parameters.

Climate (temperature, rainfall, humidity) play a vital role in the lifecycle of arthropod vectors [10,27]. As arthropods are poikilothermic, the environmental temperature is a key factor affecting the rate at which an arbovirus is able to replicate to a transmissible level within a vector [11]. Biting midges spend almost 90% of their lifetime resting while digesting blood meals and developing eggs [16,28], and the temperatures to which the

insects are exposed are therefore important when modelling vector-borne diseases. However, most models of *Culicoides*-borne diseases [1,4,10,11,16,25,27] predict the VC or R_0 using meteorological temperature rather than the actual temperature in the microclimatic environment of the vectors. Studies in Scandinavian climates have shown that microclimatic temperatures are warmer during the day and cooler during the night compared to recordings from nearby meteorological weather stations, and that the differences significantly affect the rate of virus development in *Culicoides* as well as the duration of the transmission season [29,30]. Even if the daily meteorological and microclimatic temperatures were the same on average, the presence of threshold temperatures and the non-linear relationship with temperatures above the threshold mean that the decreased speed of virus development during the night will not compensate for the increase during the day. This means that models that use meteorological temperature may not predict the correct development rates.

Culicoides imicola was previously considered the main vector for BTV transmission in southern Europe [31,32]. Recently, BTV virus was isolated from wild specimens of *Culicoides obsoletus* (Meigen), *C. scoticus* (Downes & Kettle) and specimens of the *Pulicaris sp.* [24,31,33,34]. Identifying *Culicoides* species based on their morphological characteristics is difficult. Therefore, the term 'ensemble' is here suggested to denote a group of sympatric species for which morphological identification is sometimes difficult or not possible without phylogenetic analysis [35]. Obsoletus ensemble refers to both the Obsoletus group and *C. dewulfi*, and includes *C. obsoletus*, *C. scoticus*, *C. montanus* (Shakirzjanova), *C. chiopterus* (Meigen) and *C. dewulfi*. The Pulicaris ensemble includes *C.* pulicaris (Linnaeus) and *C.* punctatus (Meigen) [35,36]. In this manuscript, we used both Obsoletus and Pulicaris ensemble abundance data to estimate the daily IB of BTV.

The objective of this study was to use a mechanistic transmission model to: i) estimate the potential daily number of IB with BTV in Denmark by including known parameter estimates and equations; ii) quantify the uncertainty associated with each parameter; iii) perform a sensitivity analysis of each parameter to quantify their impact on BTV transmission estimates.

Methods:

In our model we used four equations for the EIP, three equations for *Culicoides* daily survival rate, two temperature-dependent equations and one fixed rate for transmission from host to vector, and one temperature-dependent equation for biting rate. We considered that all equations were equally likely to illustrate the impact of temperature on virus development, vector survival, blood meal digestion or transmission from host to vector. We then ran the model with all possible parameter combinations (576). Each model combination was then run in combination with a distribution of four vector abundance and distributions of microclimatic temperature data for four potential insect habitats; in total 2304 combinations of model parameters, vector abundance and microclimatic temperatures.

Estimating microclimatic temperatures in Denmark, 2000-2016

The method for estimating the microclimatic temperatures around 22,004 Danish cattle farms is described in detail by Haider et al. (2017, 2018) [29,30], and a summary analysis of the temperatures is available in Haider et al. (2018) [30]. A brief description will follow.

Hourly meteorological parameters (temperature, solar radiation, humidity, wind speed) for the period 2000-2016 were obtained from the Danish Meteorological Institute (DMI) at 320 grid points across Denmark. The nearest grid point to each of the 22,004 cattle farms was considered to be the temperature at that farm. The type of land cover within a 500 m radius of each cattle farm was quantified using CORINE Land Cover [37]. The land cover was reclassified as dry meadow (83%), hedges (6%), wet meadow (3%), and forest (3%) [30]. Using a published microclimatic model [29,30], these meteorological parameters were converted into hourly temperatures for the period of 1st April to 31st December for each of the four different microclimatic habitats. This resulted in four different hourly temperatures for 22,004 cattle farms over the 17 years.

In the present study, we calculated six different hourly temperatures for the period of 1st April to 31st December for each habitat. These temperatures were the minimum, maximum, mean of first quantiles (> minimum and <25th percentile), second quantiles (≥25th percentile <50th percentile), third quantiles (≥50th percentile <75th percentile) and fourth quantiles (≥75th percentile) for a particular hour on the 22,004 farms over the 17-year period, resulting in six series of hourly temperatures from 1st April to 31st December. We used a national distribution of hourly temperature without considering the spatial variation. To quantify the impact of temperature, we ran our model with all six series of temperatures for each of the four habitats in all combinations with the xx model parameters setting.

***Culicoides* density data:**

A number of surveillance and research projects on trapping biting midges have been carried out across Denmark since 2007. Surveillance was conducted at 22 sites over the winter of 2007, 2008 and 2010 to identify the vector-free season. To monitor *Culicoides* in the warm season, 12 sites were selected randomly across Denmark during 2008 and 2009, and each site was sampled 29 times. Four of these 12 farms were selected and midges were collected for two nights every week during 2012, and three nights every week after this. This is part of the national monitoring of *Culicoides*, and surveillance information is updated on the website: http://www.myggetal.dk/. Surveillance data were available for the period 2012-2016. In addition, collections were taken over one night at 251 farms in 2008 and 124 farms in 2009 from different regions of the country. The Onderstepoort light trap was used to collect the biting midges. Where collections were made on more than one night, the daily mean number was used for each day, with the assumption that the trap would collect midges equally each day. Details of the counting and species-identification methods are described by Kirkeby (2012) [7] and Lassen et al. (2012) [38]. All data were available for this study.

We calculated the minimum, maximum, mean of first quantiles (> minimum and <25th percentile), second quantiles (≥25th percentile <50th percentile), third quantiles (≥50th percentile <75th percentile) and fourth quantiles (≥75th percentile) number of Obsoletus and Pulicaris ensembles separately from the weekly

distribution of trap data. We then generated a seasonal daily abundance for each of the six estimates for each species ensemble by assuming the abundance would be the same on each day of the week. Finally, we smoothed each of the 12 daily *Culicoides* abundance data by a 14-day running average and used these 12 estimates as averages of the daily vector abundance on cattle farms in Denmark over an average season.

Parameters/equations used:

The equations used in the transmission model are listed in **Table 1 and Figure S1**. Summaries of vector abundance and temperature data are presented in **Figures 1** and **2**. We did not find a quantitative description of a temperature-dependent equation for host-to-vector transmission rate for the Obsoletus and Pulicaris ensembles. The Obsoletus ensemble is considered to be a very competent vector for BTV transmission [24,33,34], so in order to quantify host-to-vector transmission rates, we used two temperature-dependent equations originally developed for *C. bolitoinos and C. imicola* [12]. As most BTV transmission models use a fixed rate of transmission from host to vector [13,39,40], we used the median value (0.071) of the range 0.001 - 0.15 used by Gubbins et al. [1] (**Table 1**). The Pulicaris ensemble comprises some of the most abundant vectors in "*imicola*-free" BTV transmission regions, and recent studies propose the species within this ensemble to be potential vectors of BTV transmission [24,36,41]. We did not want to ignore their role as potential vectors, and used a mean value of 0.0402% vector competence for BTV for the Pulicaris ensemble, which was presented in two different studies [24,33].

In this manuscript, the four different EIP equations are referred to as EIP equation I [16], EIP equation II [42], EIP equation III [42], EIP equation IV [11], and the three different daily insect survival rate equations are Survival rate equation I [42], Survival rate equation II [17] and Survival rate equation III [17]. Likewise, we refer to the two temperature-dependent equations for the rate of BTV transmission from host to vector as Host-vector equation transmission I (developed for *C. imicola)* [12,13] and Host-vector equation transmission II (developed for *C. bolitinos*) [12,13] (**Table-1**).

Mechanistic model for estimating the number of daily infectious bites by *Culicoides* insects:

We used a mechanistic model to estimate the potential number of infectious bites originate from one infectious host via *Culicoides*. This is a biological process-driven model based on the above-mentioned parameters. The model has previously been described [43], and a similar model for another vector-borne disease, *Setaria tundra,* has been described by Haider et al. (2018) [44].

The model is designed to follow daily cohorts of biting midges throughout the season at hourly temperatures estimated for the four habitats: dry meadow, wet meadow, hedges and forest. In the model, biting midges take a blood meal infected with BTV, rest until the gonotrophic cycle is completed and then successfully take a new blood meal. Completion of the EIP is solely dependent on the hourly temperature experienced by the cohort of midges each day. After the EIP is complete, we assume that the biting midges infect a new host with all subsequent bites until all vectors in the cohort are dead. We used a rate summation model in which the EIP or blood meal digestion rate is calculated hourly and summed up daily until the parasite development/blood meal digestion is complete.

The steps in the model are described below:

1. The daily survival rates for *Culicoides* midges are calcuated using the daily mean temperaure and the equation listed in **Table 1**. We assumed a maximum survival time of 60 days for *Culicodes* biting midges in Denmark with a daily maximum survival rate of 90% and minimum survival rate of 1% (Table)-1.
2. The model calculates the EIP of BTV (**Table 1**) based on successive hourly temperatures for each daily cohort and identifies the date when the biting midges in each cohort become infectious, i.e. when the EIP is complete.

3. The model calculates and identifies the dates when the vectors complete each gonotrophic cycle (**Table 1**) based on the successive hourly temperatures for each daily cohort. It is assumed that the biting midges will take a new blood meal immediately after the gonotrophic cycle is complete.
4. The model identifies the date of the IB in each cohort by comparing the dates when vectors bite after the EIP is complete. This date is then merged with information on the survival rates of biting midges to calculate how many vectors of the original cohort have survived until that day. The number of surviving vectors represents the number of new IB by the vectors in the specific cohort.
5. The model estimates the proportion of vectors that become infected while taking a blood meal. This is done seperately for the Obsoletus and Pulicaris ensembles by using two temperature-dependent formulae (for Obseoltus ensemble only) as well as two fixed rate (one for Obsoletus ensemble and one for Pulicaris ensemble; **Table 1**). This proportion is multiplied by the number of surviving vectors to estimate the total number of IB produced by the cohorts.
6. The model assumes 80% of IB will successfully infect the host by multiplying the total number of IB by a factor of 0.80 (the rate of BTV transmission from vector to host, **Table 1**).
7. The model then sums up all the IB originating from each daily cohort of vectors.
8. The model estimates the IB for the Obsoletus and Pulicaris ensembles seperately, then sums them up to estimate the total number of IB for each day.
9. For example, an IB of 10 on 1st August means that if a cohort of vectors bite an infectious animal on 1st August, 10 new animals will be infected by the cohort during its lifespan (after successful completion of the EIP). We assumed a maximum *Culicoides* lifespan of 60 days, so the last date that this cohort could infect a new host would be 1st October.

Summary of infectious bite estimates:

We used four series of hourly temperatures (mean of fourth, third, second, and first quantiles) from each of four microclimatic habitats (dry meadow, hedges, forest and wet meadow), four series of *Culicoides* data in both the Obsoletus and Pulicaris ensembles (mean of fourth, third, second and first quantiles), four equations

for the EIP, three equations for vector survival rate, and three different host-to-vector transmission rates for the Obsoletus ensemble, but only one for the Pulicaris ensemble. We ran the model using all combinations (4 Temperatures × 4 Habitats × 4 Vector abundance × 4 EIP × 3 Survival rates × 3 host-to-vector transmission rates), resulting in a total of 2304 different IB for each day of the transmission seasons for *Obsoletus* ensemble. In addition, for Pulicaris ensembles our model estimated 768 different IB for each day (as there was only one equation for host to vector used for *Pulicaris* ensemble). Thus for each day we have a total of 3072 IB estimated with different combinations.

To identify the worst- and best-case scenarios, we also ran the model with the maximum and minimum temperatures and the maximum and minimum abundance of biting midges, respectively. This gave another 144 estimated for each of the best and worst scenario (4 EIP × 3 Survival rate × 3 rate of transmission from host to vector × 4 habitats) for Obsoletus and 48 estimated for each of the best and worst scenario (4 EIP × 3 Survival rate × 4 habitats) for Pulicaris ensemble. We identified the worst case as the daily maximum value of IB for each day from 144 different estimates. Likewise, we identified the best case as the daily minimum value of IB for each day from 144 different estimates.

Since 83% of land cover around the Danish cattle farms was dry meadow, and hedges (6%) were double than the forest (3%) and wet meadow (3%), we adjusted our model output by replicating the daily IB estimates 7 times by those estimated from dry meadow temperature, 2 times with those estimated by hedges and used the complete dataset including the IB estimated with wet meadow and forest temperature in summary analysis.

Data analysis:

We calculated summary statistics to report the daily mean temperature and the 10-90th percentiles of the different microclimatic habitats and the DMI weather stations. We also did this for the number of biting vectors per day. We calculated the daily mean IB and the 10-90th percentiles from the 3,072 calculations of the daily number of IB. To quantify the sensitivity of the model to variation in each of the six different

parameters, we calculated the daily mean IB for each category of that particular parameter while allowing all other parameters to vary in all combinations. The mechanistic model for estimating the daily IB was developed in SAS version 9.4 [45] and all summary analyses and plots were performed in R version 3.4.0 [46].

Results:

Culicoides vectors in Denmark:

We identified a total of 1,463 trap collections over 1- 3 nights from 351 cattle farms across Denmark between 2007 and 2016. The mean number (10-90th percentiles) of the Obsoletus ensemble was 204 (0-488) per night, and the mean number of the Pulicaris ensemble was 142 (0-288). The number of vectors in the Obsoletus ensemble started to increase in early May, reaching a peak in July with a mean (10-90th percentiles) of 388 (0-1,140) midges per night. The number of vectors in the Pulicaris ensemble started to increase in April and peaked in June with a mean (10-90th percentiles) of 281 (1- 578) midges per night **(Fig. 1)**. We found four or five generations of Obsoletus in the annual average, with the first peak seen in May, the second in June, third in July, fourth in August and the fifth and final peak was surprisingly seen between late October and the beginning of November (**Fig. S2**). Although not as clear as for the Obsoletus ensemble, five or six generations of Pulicaris ensembles were also observed between April and November (**Fig. S2**).

Temperatures in Denmark:

The temperature data from DMI and the microclimatic temperature data are summarized in **Figure 2**. The dry meadow was the warmest microhabitat with a mean (10-90th percentile) summer (July and August) temperature of 17.9°C (12.4-24.6°C) compared to hedges 17.8°C (13.9-23.1°C), wet meadow 15.8°C (13.3-19.4°C) and forest 16.7°C (14-19.4°C). The mean (10-90th percentiles) summer DMI temperature was 17.0°C (14.6-19.6°C).

Daily infectious bites:

The estimated number of daily IB with BTV is summarised in **Figure 3**. The mean (10-90th percentiles) number of IB estimated for Denmark was 2.2 (0-8.9) per day for the period 1st April to 31st October, taking into consideration all possible combinations of parameter values. The daily mean (maximum) IB was 449 (2,899) in the worst-case scenario, while no transmission was detected in the best-case scenario.

Seasonality:

The earliest possible day when a cohort of *Culicoides* could become infected and successfully transmit BTV was in the second week of April. The last date was in the second week of September (or third week of October based on the worst-case scenario; **Fig. 3)**. While considering the median IB of all combinations, the period when vectors could become infected and successfully transmits BTV had a range of almost 3 months, starting in the third week of May and ending in the third week of August. Three peaks of host-to-vector transmission were observed: at the beginning of June, between the last week of June and the second week of July, and the final and largest peak was observed between the third week of July and first week of August. This peak of transmission from host to vector correlated with the number of Obsoletus ensemble during the same period. The mean (maximum) monthly IB with BTV was 0.30 (59) in April, 2.1 (155) in May, 4.1 (406) in June, 6.3 (616) in July, 2.4 (401) in August, 0.34 (74) in September, and 0 in October (**Fig. S3**).

Model sensitivity to parameters driving daily bluetongue virus transmission:

We compared the impact of the different parameters used in our model on estimates of daily IB with BTV (**Fig. 4**). Survival rate equation I estimated higher IB (mean: 4.8) compared to survival rate equation II (mean: 1.2) and survival rate equation III (mean: 0.69).

The mean daily IB was estimated at 3.75 by EIP equation I, 1.96 by EIP equation II, 2.1 by EIP equation III, and 1.16 by EIP equation IV. We used two kinds of equation for the rate of transmission from host to vector. Two temperature-dependent equations were based on published findings for *C. imicola* and *C. bolitoinos*, and we used these equations for the Obsoletus ensemble only. However, we also used one fixed rate of

transmission from host to vector for Obsoletus and one for Pulicaris. The number of IB by host-to-vector transmission estimated with equation I was lower than with equation II (0.21 vs. 2.5). The fixed rate for the Obsoletus ensemble estimated a mean daily IB of 1.3. The fixed rate of transmission from host to vector for the Pulicaris ensemble estimated an average of 0.41 daily IB.

When the first quantile temperature was used, the model did not estimate more than one IB per day on average. The IB estimated from the fourth quantile mean temperature was double that estimated from the third quantile mean temperature and 14.6 times higher on average compared to estimates from the second quantile temperature.

In presence of favorable temperature (when temperature is enough to accomplish the EIP) vector abundance was the most influential parameter driving the IB with BTV. The daily IB estimated from the fourth quantile mean Obsoletus ensemble was on average 17 times higher than the IB estimated from the third quantile mean Obsoletus ensemble and 408 times higher than estimates from the second quantile temperature. Estimates from the first quantile Obsoletus ensemble abundance never resulted more than one IB on average per day. For the abundance of the Pulicaris ensemble, only the fourth quantile mean biting midges estimated more than one IB on average.

The mean daily IB was 2.5 for dry meadow temperature, 1.5 for hedge temperature, 1.1 for forest temperature and 0.93 for wet meadow temperature. The EIP equation developed for BTV-9 estimated a higher IB (daily mean: 3.8) compared to the equation developed for BTV-10 (mean: 2.1), BTV-16 (1.9) and non-specific BTV (1.2).

Variation in infectious bite estimates:

We identified a large uncertainty in the estimated IB associated with different equations for the parameters. For example, on 1st July, the daily IB was 0.01 at the 10th percentile, 0.46 at the 50th percentile, 11.1 at the

362 90th percentile and 91.8 at the 99th percentile, whereas the maximum IB was estimated as 728 (**Fig. 3**). We did
363 not find a large discrepancy in the start or end of the season as estimated by the different equations (EIP,
364 survival rate, host-to-vector transmission). However, different temperatures and vector abundance resulted in
365 different transmission season durations. While the worst-case scenario shows almost 6 months of
366 transmission season, first quantile mean temperature showed around 3 months of transmission (mid-May to
367 mid-August) and first quantile mean Obsoletus showed less than 3 months of transmission (mid-May to the
368 end of August).
369
370
371 **Discussion:**
372 Our estimation is based on the assumption that all the available equations used in BTV modelling are equally
373 good at estimating the daily IB. Our model generated 3072 different estimates by combining different
374 parameter estimates of IB with BTV in Denmark for each day of the transmission season (1st April to 31st
375 October). The worst-case scenario predicted a very high average of more than 400 daily IB for the entire
376 season (April-October), with a maximum daily value of 2,899. The worst-case scenario was identified as the
377 highest number of IB estimated from the highest temperature recorded every hour over 17 years from any
378 part of the country along with the highest number of biting midges recorded that week and a combination of
379 the remaining equations for daily survival rate, EIP, blood meal digestion period and host-to-vector
380 transmission rate that gave the highest number of IB on specific day. The best-case scenario showed that no
381 transmission would be possible in Denmark, and was identified as the lowest number of IB estimated with the
382 lowest hourly temperatures over 17 years from any part of the country, leading to a dataset where virus
383 development was never possible within the lifespan of the vector. The mean number of IB for all
384 combinations is above two for the entire season, indicating that BTV can become epidemic if introduced,
385 especially taking into consideration that an infected host will remain infectious for approximately 3 weeks [47].
386

Daily IB refers to the ability of the vector population to transmit a pathogen to the host population [16] and is different from the basic reproduction rate or R_0 in that the period of viremia is not accounted for. The period of viremia in naturally infected animals varies for different strains of BTV with a range of 14-63 days and a mean of 20.6 days [47,48]. This long infectious period could result in a large outbreak with the IB estimate from our model. Our estimate therefore shows that if BTV is introduced into the country, local spread is likely to occur. This finding is in agreement with empirical observations of BTV outbreaks in 2007 and 2008 in Denmark, when a large number of animals in each farm were infected with BTV [49].

Among the parameters driving the daily IB, the largest variation was observed with changes in the number of vectors. An increasing of one quantile mean number of Obsoletus ensemble resulted in a daily IB that was 17 times higher. This is due to the large variation observed in the population of Obsoletus ensemble. We found several generations of Obsoletus and Pulicaris ensembles during an average season. The peak of each generation coincided with the peak of daily IB. In earlier studies, daily R_0 estimation based on observed and predicted Obsoletus complex coincided with a peak in the population of vectors [6]. The Pulicaris ensemble population peaks earlier in the spring, and there was a small peak in the daily IB at beginning of May primarily due to this. Pulicaris is not considered to be a competent vector for BTV transmission, although they are abundant in the "non-imicola" region where BTV was detected [50], and recent studies indicate that they play a role in BTV transmission [24,33]. In studies from southern and central Europe [6,34], the Pulicaris ensemble population only constitutes a small fraction of the Obsoletus population, yet we found that in Denmark the Pulicaris ensemble population was around half that of the total population of Obsoletus over the entire period. In general, Pulicaris are considered to be more abundant in Scandinavia than in central and southern Europe [51].

Temperature plays a critical role in driving many other parameters including the EIP, survival rate, biting rate of vectors and host-to-vector transmission rate. In the best case scenario we used the minimum temperature each hour to estimate the infectious bites of BTV which predicted transmission was not possible at any day.

There was no transmission because virus development was not completed within the lifespan of the vectors. In a situation like this the number of insects is irrelevant; BTV transmission will not be possible despite high abundance of vectors. The model that used the first quantile mean temperature estimated a very low daily IB because virus development was not completed during the lifespan of the vectors. One of the survival rates proposed by Gerry and Mullens (2000) estimated a very low daily IB, because only a small proportion of vectors survived until the EIP was complete. Higher daily IB were estimated in dry meadow compared to other habitats, simply because the temperature was higher than at any other resting site.

In an earlier study from Denmark, Græsbøll et al. (2012) showed that the temperature and seasonality of vectors determines the period during which an incursion of BTV could lead to epidemic spread[48]. Furthermore, the authors concluded that within the transmission season, the number of affected animals will depend on the temperature and vector abundance, behaviour and ability to locate hosts [48]. Our findings are in agreement with this. If the temperature remains favourable, the size of outbreaks will depend on the vector population. A generation of *Culicoides* will generally take 4-5 weeks to reach a peak in their abundance.

During the 2008 BTV outbreak in Denmark, most of the cases were identified in late autumn (generally between September and October, while the last case was detected on 17th November) [5]. The date of some infections could possibly be earlier due to a delay in outbreak identification, and it has been unclear how BTV can be transmitted at the low autumn temperatures found in Denmark. Our model used estimated microclimatic temperatures of potential vector habitats and showed that the last date when a cohort of vectors could be infected and be able to infect new hosts after completion of the EIP was in the second week of September (early autumn). Considering the maximum lifespan of *Culicoides* is 60 days, our model showed that a successful transmission from vector to host is possible even in mid-November (late autumn) in Denmark.

A larger variation in IB was estimated by temperature-dependent equations for different parameters. The largest variation was estimated from the equation used for the daily survival rate of *Culicoides*. The mean IB estimated from survival rate equation I was 3.5 times higher than equation II and 6.1 times higher than for survival rate equation III. Such a considerable difference for the same parameter can lead to a very uncertain output of the R_0 model and can create difficulties when planning prevention and control strategies. Furthermore, the IB estimated by the host-to-vector equation was just 0.27, while another equation estimated an average of 3.1, which is 11.4 times higher.

Denmark experienced BTV outbreaks in two consecutive years in 2007 and 2008 [5]. Our model estimated a wide range in the predicted number of IB, and a number of combinations showed no transmission was possible in Denmark. As BTV already occurred in the country, the combinations of equations that resulted in no transmission being possible must be incorrect. Since we used observed vector abundance and predicted microclimates, the combination of equations that resulted in no transmission being possible are likely to be the survival rate equation III, host-to-vector transmission rate I and EIP equation I. We suggest that due care should be taken when using combinations of these equations in modelling the transmission of BTV in northern Europe.

Vector-borne diseases are temperature-dependent. Using one equation for a parameter can result in a very different estimate than when another equation is used for the same parameter. Therefore, one equation could underestimate or overestimate the transmission potential. For example, survival equation I [15] estimated a mean daily IB of 4.8 and a transmission season of 5 months. However, daily survival rate equation III [17] estimated a mean IB of 0.69 with a transmission season of less than 2 months. We do not know which equation most realistically captures the actual impact of temperature on vector survival.

In our model, we estimated each day during a season when a cohort of *Culicoides* could become infected and successfully transmit BTV. This means that if the first week of July was identified as having the highest

estimated number of IB, the highest number of insects would become infected and be able to transmit the infection to the host over the following 60 days (we considered 60 days to be the maximum lifespan of *Culicoides* midges). This could therefore be as late as first week of September. If prevention and control measures are sought, attention should be paid to the periods when the highest number of insects can be infected. Our model shows that July is the month when the largest number of insects are likely to be infected with, and successfully transmit, BTV.

We used four equations for the EIP, three equations for the daily survival rate, two equations for the host-to-vector transmission rate and one equation for biting rates. There might be more equations available for each of the parameters that we did not identify. Inclusion of all possible and relevant equations will provide better estimates for BTV transmission potential.

Conclusion:

In the worst-case scenario, the transmission season lasts around 6 months (mid-April to mid-October), with a maximum daily IB of 2,899. The best-case scenario in our model showed no transmission was possible. The mean (10-90th percentiles) IB with BTV was 2.2 (0-8.9) per day over the transmission period. When taking into consideration the long infectious period of BTV, it is very likely that local spread will occur in Denmark if the virus is introduced to an average herd. We identified a large uncertainty associated with the number of IB estimated by different equations, including those for daily survival rate and host-to-vector transmission rate. We found temperature and the number of vectors to be the most influential factors in determining the daily number of IB. Both the vector abundance and the temperature vary from year to year as well as spatially within Denmark. Therefore, we suggest using a range of temperatures and *Culicoides* abundance, and estimating R_0 or daily IB as a distribution rather than as a point estimate. Our model showed that the effective BTV transmission period is long in Denmark, and vectors infected as late as mid-September (early autumn) can successfully transmit BTV to a new host in mid-November (late autumn).

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Author's contributions Statement:

NH led model development, data analysis and the manuscript writing, LJK helped to interpret the data analysis and provided critical input in manuscript writing, SAN and HS was involved in Culicoides data collection and provided critical input in manuscript draft, and RB planned the original study, helped with data analysis, development of model and critically reviewed the manuscript. All authors reviewed and approved the final draft.

Competing financial interests: There is no known competing financial interest.

626 **Table:**

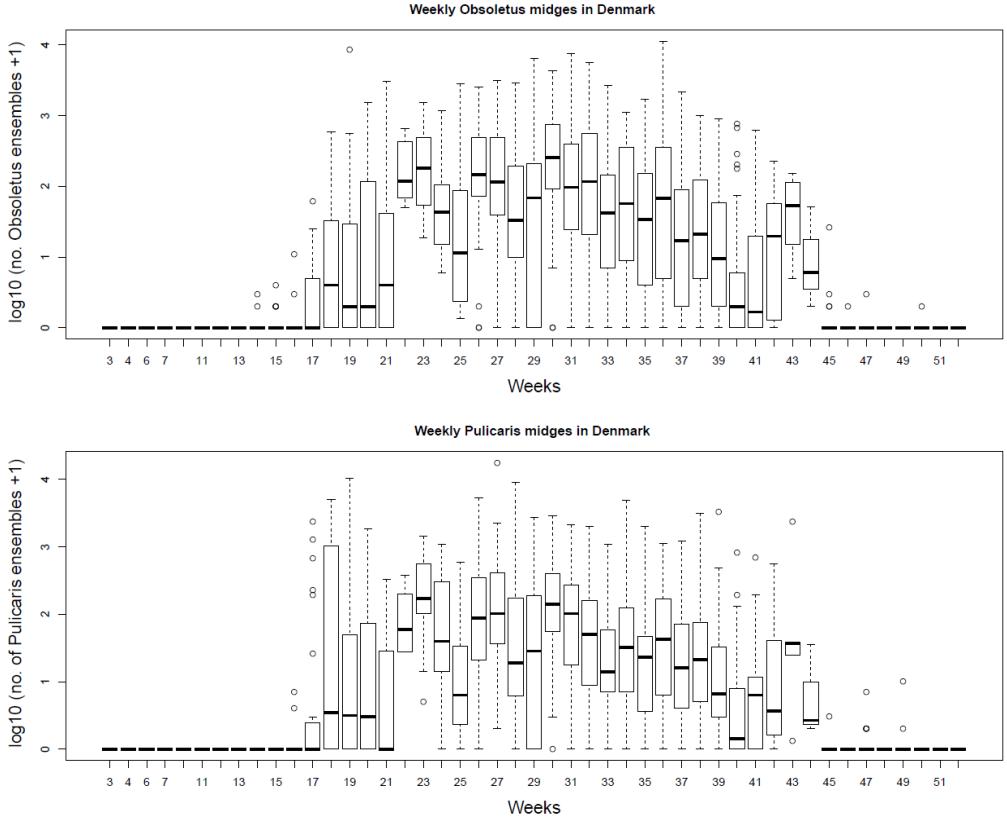
627 **Table 1: Parameters used in modelling bluetongue virus**

Parameters	Equations	Sources/References	Name in the manuscript
Survival rate of *Culicoides* biting midges	1-(0.015*exp(0.063*Temp))	Wittmann et al. (2002) [21]	Survival rate equation 1
	EXP (-1/(111.84*EXP(-0.1547*Tmean)))	Gerry & Mullens (2000) [17]	Survival rate equation 2
	1-(0.009* exp (0.16*Tmean))	Bessell et al. (2016) [52] Gerry & Mullens (2000) [17]	Survival rate equation 1
Extrinsic Incubation Period	((0.0003T(T-10.4)) (BTV)	Mullens et al. (2004) [16]	EIP-1
	0.0069T -0.0636 (BTV 10)	Wittmann et al. (2002) [21]	EIP-2
	0.0113T -0.1419 (BTV 16)	Wittmann et al. (2002) [21]	EIP-3
	0.019 *(T - 13.3) (BTV 9)	Wilson & Mellor (2009) [19] Carpenter et al. (2011) [11]	EIP-4
Biting rate (blood meal digestion)	$0.0002T(T-37)(41.9-T)^{1/2.7}$	Mullens et al. (2004) [16]	Biting rate
Rate of transmission from host to vector	0.0003699 exp(0.1725T) *(C. imicola)* (Used for Obsoletus ensemble only)	Paweska et al. (2002) [12,13]	Host to vector 1
	0.005465 exp(0.159T) (*C. bolitinos*) (Used for Obsoletus ensemble only)		Host to vector 2
	Fixed number (Used for Obsoletus ensemble only)	0.071 (Median of the distribution used by Szmaragd et al., 2009) [17,25]	Host to vector 3
	Fixed number (Used for Pulicaris ensemble only)	0.04025 (Mean of the tested Pulicaris vectors for BTV in two studies [24,33] Carpenter et al., 2006 Hoffmann et al., 2009)	Host to vector
Rate of transmission from vector to host	0.80	O'Connell (2002) [23]	Vector to host

628

629 **Figures**

Fig 1: Boxplot of weekly *Pulicaris* and *Obsoletus* ensemble abundance in Denmark collected in 2007-2016. The bottom and top of the box indicate the first and third quartiles; the band inside the box is the median. The dots outside the box are outliers.



637

638 **Fig 2:** Hourly microclimatic temperatures of four potential insect habitats around 22,004 Danish cattle farms

639 and the Danish Meteorological Institute modelled temperature in Denmark.

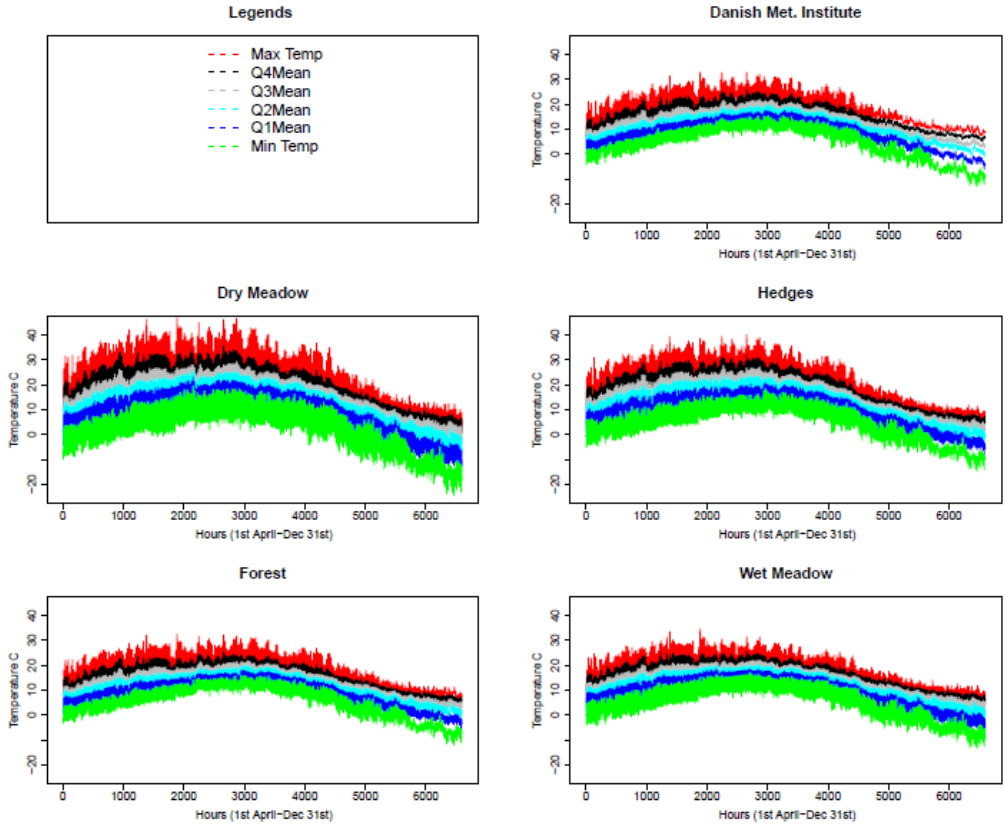


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Fig 3: The daily number of infectious bites (IB) with bluetongue virus (BTV) in Denmark. Four different microclimatic temperatures were chosen from 2000-2016, as well as weekly vector density data from 1,453 trap collections across Denmark, four different equations of extrinsic incubation period, three equations of vector survival rate and three sets of host-to-vector BTV transmission rates. The worst-case scenario estimated a maximum of 2,899 daily IB, whereas the best-case scenario estimated no transmission being possible in Denmark. We identified several peak periods of infection.

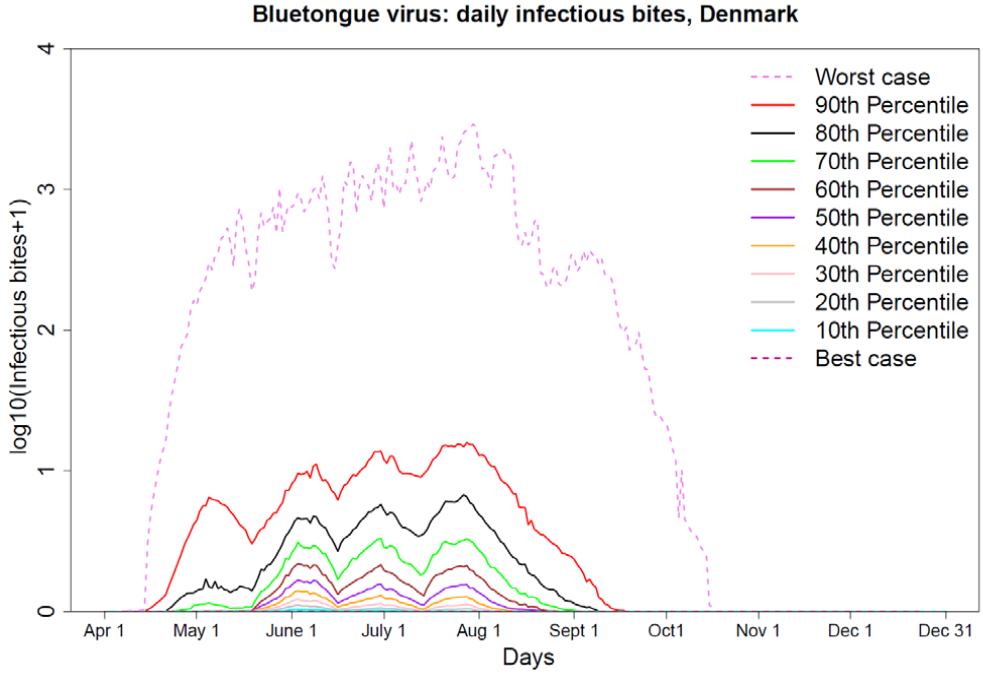
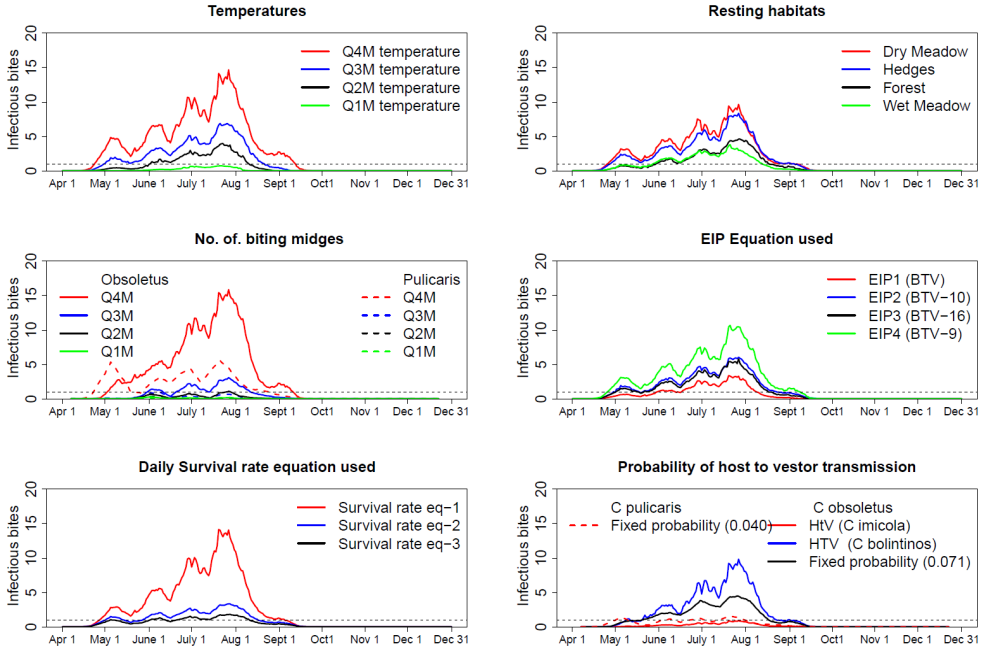


Fig 4: The relative impact (sensitivity) of different parameters used in estimating the daily infectious bites with bluetongue virus in Denmark.



Additional/supplementary information:

Fig S1: The relationship between temperature, pathogen development rate, daily survival rate, blood meal digestion rate and host-to-vector transmission rate in *Culicoides.* Maximum survival rate was set as 90% and minimum survival rate was set as 1%.

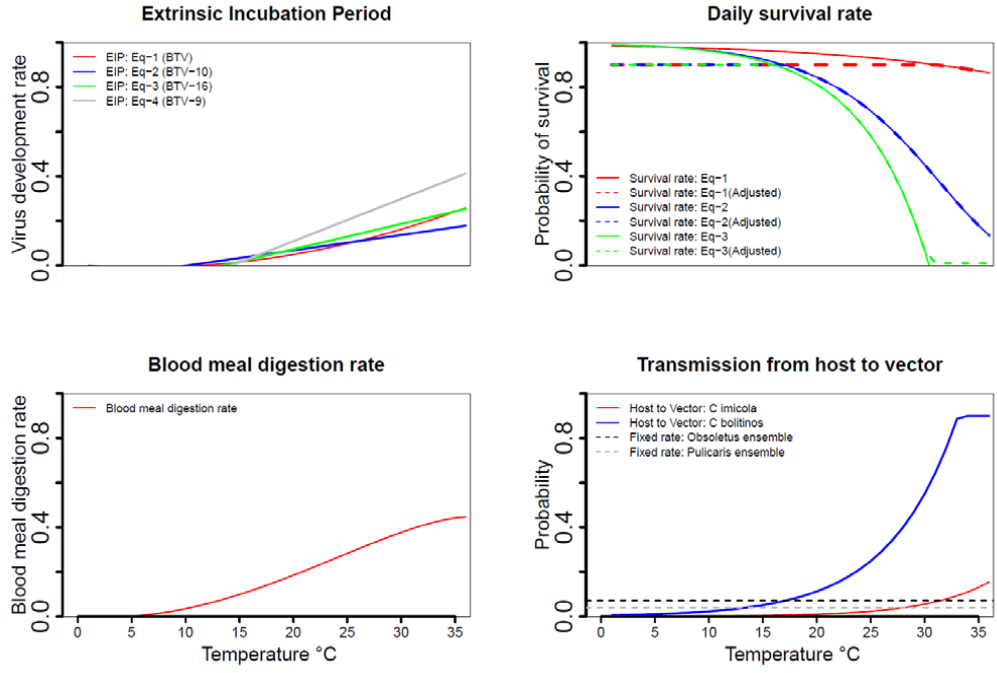


Fig S2: The summed number of biting midges from the Obsoletus and Pulicaris ensembles in Denmark. Data have been smoothed with a 14-day running average.

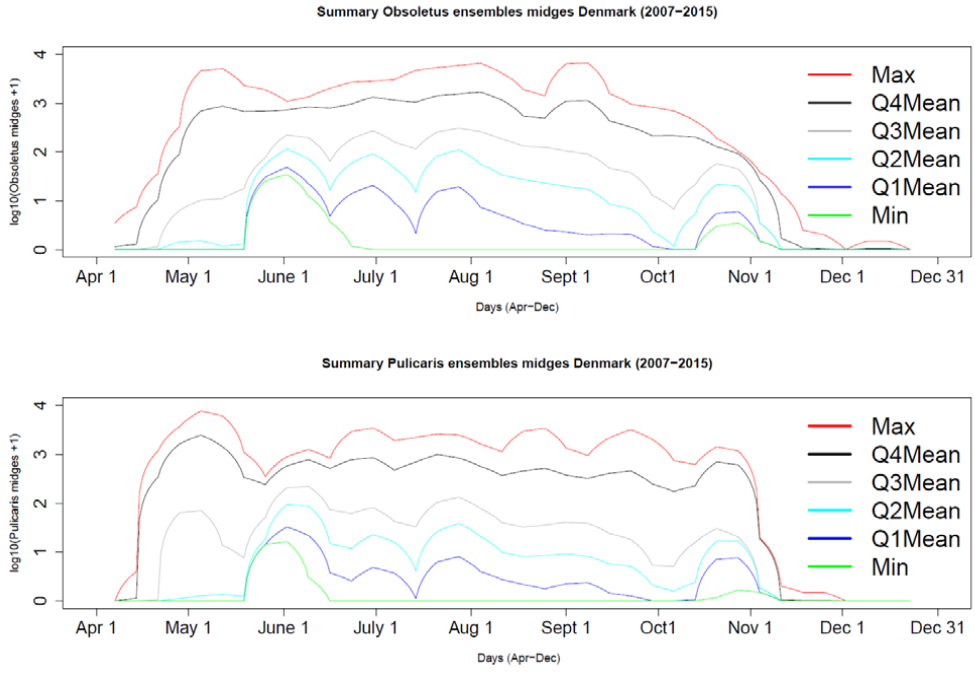
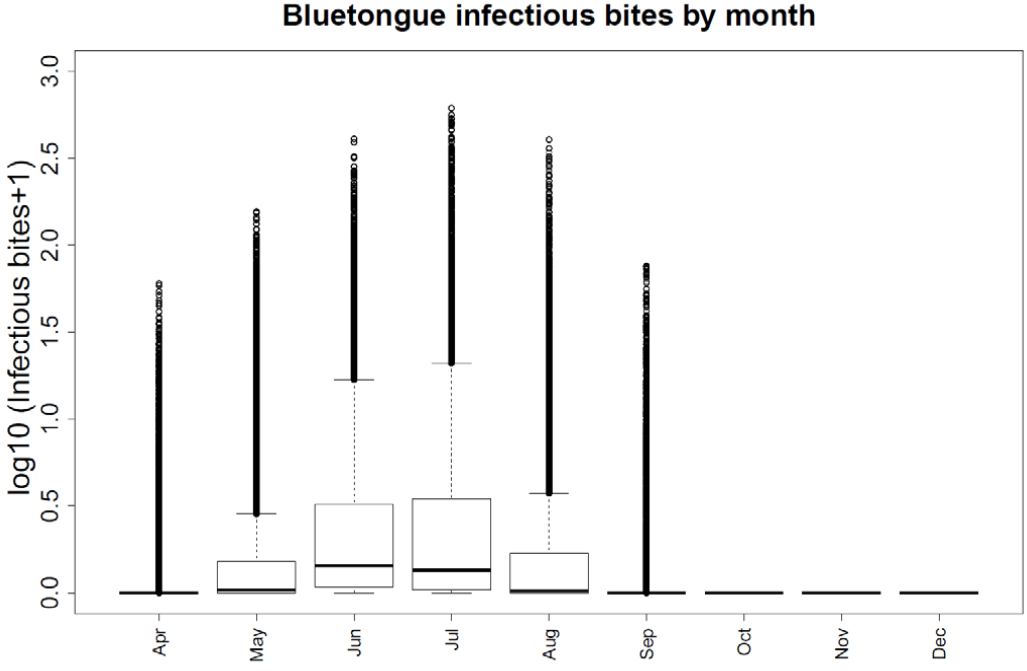


Fig S3: The boxplot of daily infectious bites (IB), estimated as the date when a cohort of *Culicoides* could become infected and successfully transmit the BTV virus in Denmark. The highest number of IB was recorded in July. The bottom and top of the box indicate the first and third quartiles; the band inside the box is the median. The dots outside the box are outliers.



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Manuscript IV

The annual, temporal and spatial pattern of *Setaria tundra* outbreaks in Finnish reindeer: a mechanistic transmission model approach

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Abstract

Background:

In northern Finland (Lapland), reindeer are reared as semi-domesticated animals. The region has a short summer season of 2-3 months, yet reindeer are infected with the mosquito-borne filarioid parasite *Setaria tundra*. The infection causes peritonitis and perihepatitis, which cause significant economic losses due to the reduced body weight of infected animals. The objective of this study was to: 1) describe the spatial and temporal pattern of outbreaks in three different areas across Finnish Lapland and 2) construct a temperature-driven mechanistic transmission model to quantify the potential role of temperature on the occurrence of *S. tundra* outbreaks and on the intensity of the worm transmission in reindeer in general.

Methods:

We developed a temperature-driven transmission model able to predict the number of *S. tundra* potentially transmitted from an infectious reindeer. We applied the model to the years 2004-2015, and compared it to the proportion of reindeer whose livers were condemned due to *S. tundra* infection at the time of slaughter.

Results:

The mean proportion of liver condemnation increased in reindeer slaughtered in late autumn/winter compared to earlier dates. The outbreaks were geographically clustered but there were no fixed foci where outbreaks occurred. Larger outbreaks were recorded in the southern regions of reindeer-herding areas compared to the central or northern parts of Lapland. Our model showed that temperatures never allowed for transmission of more than a single generation of *S. tundra* each season. In southern (Kuusamo) and central (Sodankylä) Lapland, our model predicted an increasing trend from 2004 to 2015 for both the duration of the effective transmission

period of *S. tundra* ($p<0.001$) and for the potential number of L3 *S. tundra* larvae being transmitted from an infectious reindeer ($p<0.001$).

Conclusions:

The effective transmission period for *S. tundra* in reindeer is very short in Lapland, but it increased over the period studied. Only one generation of *S. tundra* can be transmitted in one season among reindeer in Lapland. Increasing temperatures may facilitate a range expansion and increasing duration of effective transmission period for *S. tundra*.

Keywords: Setaria tundra, spatial, temporal, outbreaks, reindeer, Finland, microclimatic temperature, climate change

Introduction

Reindeer (*Rangifer tarandus tarandus*) husbandry is an integral part of the Arctic culture in Finnish Lapland [1]. An increase in outbreaks of mosquito-borne filarial *Setaria tundra* infection has been documented in Finnish reindeer in recent years [2]. The disease is associated with peritonitis, perihepatitis and poor body condition [2–4], and has therefore had a negative impact on the welfare of reindeer [5]. A correlation has been found between the number of adult worms in the abdominal cavity and the degree of peritonitis/perihepatitis in slaughtered reindeer [6]. At least three large outbreaks of *S. tundra* in ungulates has been documented in Finland [2]. The first outbreak occurred in 1973 with a high mortality rate – the Finnish reindeer population decreased from 140,000 to 98,000 [4]. In addition, 1973 was one of the warmest years recorded in Lapland over the last century [2,3,6]. In another warm year, 1989, another large outbreak was recorded in moose (*Alces alces*) [4,7]. The most recent large outbreak in reindeer was recorded in 2003 in the southern part of the Finnish reindeer-herding area [2,3]. These three outbreaks were all associated with relatively warm summers, and a relationship between global climate change and increasing *S. tundra* outbreaks in Finnish reindeer has subsequently been suggested [4]. Studies also showed a correlation between higher mean temperatures of the two successive summer seasons and *S. tundra* outbreaks in Finland [4]. While many other studies on vector-borne diseases have suggested a similar correlation between increasing temperatures (global warming) and disease outbreaks [8,9] (e.g. bluetongue virus outbreaks in Europe [10] and Zika virus outbreaks in the Americas [11]), the exact mechanism of how increasing temperatures cause such outbreaks is not well described. Such mechanisms could, however, include: i) an increase in the duration of the annual transmission periods, allowing more generations of the pathogens, ii) faster pathogen development time in vectors, iii) increased vector abundance, hence increased mosquito-biting rates. In this study, we explore how increasing temperatures may have affected the annual accumulated transmission of the infective stage of *S. tundra* larvae to reindeer hosts.

Successful transmission of *S. tundra* depends on the number of vectors, the proportion of infectious hosts and environmental temperatures (**Fig. S1**). After ingesting microfilaria from an infected reindeer, the

mosquitoes must survive long enough for the microfilaria to develop into infective larvae stage 3 (L3). The development time from microfilaria to L3, known as the Extrinsic Incubation Period (EIP), depends on the environmental temperature to which the mosquitoes are exposed [12,13]. There are no available data on the relationship between temperature and development rates of *S. tundra* L3 in mosquitoes, though studies on the mosquito-borne filarial worms *Dirofilaria immitis* and *D. repens* have generally found 14°C to be the lower threshold temperature for development into L3 [14–16]. Laaksonen et al. (2009) found that microfilaria develop to the infective L3 stage in mosquitoes over approximately 14 days at a mean temperature of 21°C, whereas development was not completed at a mean temperature of 14.1°C in Finland [3]. However, little is known beyond these two temperature records. After completion of the EIP, mosquitoes transmit the L3 to a new susceptible host, where L3 develops into an adult worm over approximately 4 months [3]. The adult male and female worms mate to generate the offspring – microfilaria [3]. Temperature is the key environmental parameter that affects most mosquito lifecycle events including survival rate, biting rate and worm development in infected mosquitoes. It is thought that the microclimatic temperature at the vector resting sites has an important impact on the transmission of vector-borne diseases in northern Europe [12,17]. High microclimatic temperatures can expedite the virus development time in insects and increase the duration of the transmission season in Scandinavian climates [12,17]. The reindeer-herding regions of Finnish Lapland have cool and very short summers (around 2-3 months), but regular, small *S. tundra* outbreaks have recurred here since 2003 [2–5].

There are 54 reindeer cooperatives in Finland [1], all of which are members of the “Reindeer Herders Association (Paliskuntain yhdistys)”, under the Ministry of Agriculture and Forestry [1]. The majority of grassing areas adhere to political, administrative or geographical boundaries. Reindeer from different cooperatives often mix while grazing in adjoining areas, as there are no physical barriers between cooperatives, except in the northernmost reindeer herding area. There are usually several herds of animals (~ 2-4) in the same cooperative, and herd owners rear the reindeer as semi-domesticated animals (under the rules of cooperative), allowing them to mix with reindeer from other owners within the cooperative. The owners provide

supplementary feed during winter [1]. There is an autumn round-up, when animals selected for breeding are treated with the antiparasitic drug Ivermectin and released. Most of the reindeer are slaughtered from September to December, but a small proportion may be slaughtered as late as the following April. Of the slaughtered reindeer, 80% were born the same year and 20% are old – usually around 8-9 years old and approaching the end of their reproductive life [6]. Most of the reindeer (~70-80%) are slaughtered in officially approved abattoirs and the rest are slaughtered privately at home by the owners [6,18]. Meat inspection has occurred regularly since the 1980s [6,18], while data on liver condemnation due to *S. tundra* and other causes (Onchocerca, general inflammation, arthritis etc.) have only been recorded precisely since 2004.

Aedes spp. and to some extent *Anopheles spp.* mosquitoes are the main vectors responsible for the transmission of *S. tundra* across Finnish reindeer-herding areas [3]. Unfortunately, the abundance and spatial distribution of these mosquito species are not well documented. Some estimates suggest that reindeer can be exposed to attacks by approximately 8,000 mosquitoes an hour during some periods [19]. In the Kuusamo region, as many as 426 mosquitoes were caught in hand nets per minute during the first week of August (data used in this study) [2]. *S. tundra* microfilaremia varies throughout the year in reindeer, with the peak period from mid-June to late August [5]. A previous study showed that the peak abundance of microfilaremia in reindeer and peak mosquito activity in Finland coincided [3].

The objective of this study was to: 1) describe the spatial and temporal pattern of outbreaks at three different latitudes in northern Finland; 2) construct a temperature-driven mechanistic transmission model to quantify the potential role of temperature in the occurrence of *S. tundra* outbreaks and the intensity of the worm transmission in reindeer in general.

Methods:

Grouping of cooperatives:

We grouped cooperatives close to three different weather stations in Kevo (north), Sodankylä (central), and Kuusamo (south) in order to calculate the mean proportion of organ condemnations and to estimate the worm burdens with a model based on meteorological data from each of the three stations (**Fig. 2**). The northern region included cooperatives 1, 2, 3, 4, 5, 6, 7, 8 & 10, the central region included cooperatives 15, 16, 17, 18, 19, 21 & 22, and the southern region included cooperatives 24, 25, 26, 35, 36, 37, 38, 42, 43, 45, 46, 48 & 51.

Liver condemnation due to S. tundra infection in reindeer:

We collected the reindeer meat inspection data from the slaughterhouses for the period 2004-2015. Meat inspection and hygiene control are conducted at slaughterhouses by the regional state administrative agencies of Finnish Lapland [18]. The meat inspection procedure has previously been described in detail [6,18]. In short, the degree of liver damage caused by *S. tundra* is measured on a scale of 0-3. There is a correlation between the number of adult worms living in the abdominal cavity of the reindeer and the condition of their livers [6]. We considered organ changes with local fibrin formation on the surface of the liver or peritoneum resulting in condemnation the whole organ (liver) (scale 2-3) as *S. tundra* infection [6]. We calculated the prevalence of *S. tundra* in reindeer as the proportion of animals whose whole livers (were condemned due to *S. tundra* compared to the total number of reindeer inspected at the slaughterhouse.

The slaughter data did not contain the age of the reindeer, so we could not distinguish which animals were born just prior to the preceding transmission season or which animals had lived through one or more transmission seasons.

Meteorological temperature data:

We collected data on the daily maximum, minimum and mean temperatures from the Finnish Meteorological Institute (FMI) for the period 1979-2015. Data were recorded at three weather stations located in three different

regions. We converted the daily maximum and minimum temperatures into hourly temperatures using a simple sinusoidal distribution as described by Parton, & Logan (1981) [20].

Microclimatic temperature data:

Microclimatic temperature is considered to be useful for estimating vector-borne disease transmission [12,17,21], but these data were not available from the study areas. A recent study of the Danish climate concluded that microclimatic habitats were on average 3.5–5°C warmer at midday than the meteorological recorded temperatures, but 1–3°C cooler at midnight [22]. In accordance with this finding, we estimated the microclimatic temperature by adding 3.5°C to the daily maximum temperature and deducting 1°C from the daily minimum temperature, then converted them to hourly temperatures using a sinusoidal distribution [20].

Mosquito abundance data:

Few data on vector abundance are available for northern Finland. Laaksonen et al. (2009) collected mosquitoes using a hand net for 60 seconds every week during summer 2004 in the Kuusamo region of Finland [2] and identified three genera (*Aedes spp*., *Anopheles spp.* and *Culiseta spp*.) [2]. In the present study, we used the data from Laaksonen et al. (sum of *Aedes spp.* and *Anopheles spp.*) to estimate mosquito abundance. We used a running average of 30 days to smooth the distribution (**Fig. S2**). With no other data available, we assumed the smoothed vector abundance recorded in 2004 would be representative of all years in order to capture the main seasonal trend. Any variation in vector abundance from year to year was thus ignored in the analysis of transmission (**Fig. S2**).

Microfilaria density data:

We used published *S. tundra* microfilaria data monitored at Oulu Zoo in 2004[5]. In March 2004, three male and four female reindeer were relocated from Kuusamo to the Oulu Zoo area and were naturally infected with *S. tundra*. The microfilariae were monitored weekly for 1 year by collecting blood from a jugular vein, as described

by Laaksonen et al. (2009) [5]. To obtain a daily observation, we calculated a 30-day running average of the microfilaria data, again assuming that the microfilaria data recorded in 2004 were representative of all years. Any variation in microfilaria density from year to year was therefore ignored in the analysis of transmission (**Fig. S2**).

Mechanistic model for estimating the number of worms transmitted from an infectious reindeer:

Terminology used:

Extrinsic Incubation Period (EIP): the temperature-dependent time interval between a mosquito receiving blood meals infected with *S. tundra* microfilaria to the microfilaria developing into infective L3 larvae.

Duration of the gonotrophic cycle: the interval between two successive blood meals taken by a mosquito.

Daily survival probability: here the survival of mosquitoes is assumed to depend only on daily mean temperature. We used a mathematical equation in which the daily survival rate of mosquitoes was dependent on temperature [23], and restricted the daily survival rate to a maximum of 90%. We considered the maximum lifespan of mosquitoes to be 60 days.

Effective period for S. tundra transmission from host to vector: the number of days in a year when mosquitoes receive an infected blood meal (with microfilaria of *S. tundra*) and survive long enough for the ingested worms to develop into L3 larvae that can be transmitted to a new host.

We developed a a mechanistic model to estimate the potential number of *S. tundra* L3 transmitted via vectors from one infectious reindeer to other reindeers during one season. In this manuscript, we call this as the potential number of L3 transmitted. We assumed that the infectious reindeer would be present throughout the year and that the reindeer were bitten daily by a number of mosquitoes estimated from the smoothed observed data from 2004. When the mosquitoes became infectious (i.e. microfilaria became L3), they would transmit all larvae to a susceptible reindeer during the next blood meal.

198 The model is designed to follow cohorts of biting mosquitoes each day throughout the season at the temperatures
199 recorded by the FMI weather stations in the three different regions (south, central and north). In the model, the
200 daily temperature is the only parameter governing the daily survival rate, vector biting rate and worm
201 development rate (EIP).
202
203 In the model, mosquitoes take a blood meal infected with *S. tundra* microfilaria, rest until the gonotrophic cycle
204 is completed and then successfully take a new blood meal. At each bite, mosquitoes consume 0.005 ml blood
205 [24] with the daily density of microfilaria recorded in 2003-2004 [5]. Completion of the EIP is solely dependent
206 on the hourly temperature experienced each day by the cohort of mosquitoes. After the EIP is completed, we
207 assumed that the mosquitoes would transmit all the L3 larvae during the first bite, and that subsequent bites
208 would therefore not be infectious. We developed a rate summation model in which the EIP or blood meal
209 digestion rate is calculated hourly and summed up daily until the parasite development/blood meal digestion is
210 complete. The three temperature-dependent equations used in the model are listed in **Table 1**.
211
212 The steps in the model are described below:
213 1. The model follows a daily cohort of vectors. A cohort is defined here as the number of vectors biting an
214 infectious reindeer host on a given day. The model follows this cohort until all vectors are dead, with the
215 maximum vector survival set to 60 days. The model runs for one cohort at a time, starting with the
216 cohort biting the first day of the selected time period and moving successively through the days of the
217 remaining time period. During each run, the model calculates three different events (daily survival rate,
218 EIP and biting rate) in the life of each cohort.
219 2. Based on the successive average temperature for each daily cohort, the model calculates the number of
220 mosquitoes surviving to the following day for a maximum period of 60 days (**Table 1**).

3. The model calculates the EIP of *S. tundra* (**Table 1**) based on the successive hourly temperatures for each daily cohort, and identifies the date when the mosquitoes in each cohort become infectious, i.e. when the EIP is completed.
4. The model calculates and identifies the dates when the vectors complete each gonotrophic cycle (Table 1) based on the successive hourly temperatures for each daily cohort. It is assumed that the mosquitoes will take a new blood meal on the same day that each gonotrophic cycle is completed.
5. The model identifies the date of the infectious bite in each cohort by comparing the dates when vectors bite with the EIP. This date is then merged with the information on survival rates to calculate how many vectors of the original cohort have survived until that day. The number of surviving vectors represents the number of new infectious bites by the vectors in the specific cohort.
6. The model gives three estimates: 1) the date the cohort became infected, 2) the date that bites from the cohort result in infection, 3) the number of infectious bites produced by the cohort each day. Based on the microfilarial density in reindeer on the date the vector became infected, the model can estimate how many L3 larvae are transferred on the date the vectors give the infectious bites.
7. When all the daily cohorts are processed in the model, the number of infectious L3 transmitted from each cohort are summarized by date, giving the total number of L3 transmitted per day throughout the season and originating from one infectious reindeer. For example, a cohort of mosquitoes may complete eight gonotrophic cycles in their lifetime, but if it is too cold for the EIP to complete within the lifetime of the cohort, the mosquitoes will not contribute to *S. tundra* transmission. However, if the EIP is completed between the fifth and sixth gonotrophic cycles, the surviving mosquitoes of that cohort will transmit L3 larvae when they take their sixth blood meal. In this case, the model further assumes that there would be no worms left to transmit during the seventh and eigth blood meals. This differs from other vector-borne infectious disease models (e.g. the basic reproduction rate model for Bluetongue and Schmallenberg virus [25,26]), in which all bites are infectious after the EIP is completed, but the virus load is not quantified.

8. We considered that each worm would contribute to detectable damage and potential condemnation at the slaughterhouses 75 days after infecting a reindeer based on observed onset of ogran condemnation on slaughterhouses

Data analysis:

We estimated the weekly and monthly mean proportion of liver condemnation due to *S. tundra* using slaughterhouse records for all cooperatives in the three regions (southern, central and northern parts of Lapland). For each cooperative, we calculated the mean annual proportion of liver condemnation and the average condemnation over 12 years (2004-2015). The estimates for each cooperative were plotted on a map in QGIS [27]. We plotted the maximum weekly proportion of liver condemnation at each region against the average cumulative daily number of L3 estimated by the model to be transmitted from an infectious reindeer for each of the three regions.

We selected maximum weekly liver condemnation because our model is based on the assumption that infectious reindeer are present at the beginning of and throughout each season. But the model does not take into account how many infectious reindeer are present as such data were not available. Proportion of liver condemnation will only be high when both the transmission potential is high and the number of infectious reindeer is high. Herds with no or a small proportion of infectious reindeer during the season would end up with very no or low condemnation rates even if the model predicts a high transmission potential. By selecting the maximum weekly condemnation rate we insured that the weekly slaughtered reindeer originated from herds where infectious reindeer were actually present during the transmission season thus allowing condemnation rates to be comparable with the model predicted transmission potential.

We performed a Mann-Kendall (M-K) test [28] to identify trends in the effective transmission period in the time series data (1979-2015) and the potential number of L3 transmitted, then reported the p- and tau values. To identify potential correlations between the duration of the transmission season and the total potential worm

burden each year, we performed a correlation coefficient test between two outputs of our model: the effective period for *S. tundra* transmission and the potential worm burden for the period 1979-2015.

Technically, 20% of reindeer could carry the infection from the previous year, as only 80% of animals are dewormed during autumn. We therefore identified the years when liver condemnation was higher than 20% to assess whether condemnation was due to transmission for that particular year and compared it with and the years when model predicted a higher number of L3 transmitted (≥100 worms) (Sensitivity). Our model predicted a higher number of L3 transmitted per infectious reindeer (>100 worms) [6] in the southern and central regions. . We also identified the years when our model predicted no/very low worm burden (<100) and slaughterhouse data showed a liver condemnation rate under 20% (Specificity). We performed a chi square test to identify the diffence between condemnation on different dates or in different cooperatives, and reported the p-value.

Results:

We obtained data for 393,161 reindeer slaughtered over 2,349 days between 2004 and 2015 at different slaughterhouses in Lapland, with a mean of 169 (range:10-2,720) slaughtered animals each day. Among the slaughtered animals, 82,474 were in the southern region, 135,628 were in the central region and 175,059 were in the northern region. The livers of 11,161 (13.5%) animals were condemned due to *S. tundra* infection in the southern region, 4,295 (3.1%) in the central region and 2,776 (1.5%) in the northern region.

The proportion of condemnation in reindeer due to S. tundra infection:

The mean proportion of liver condemnation in reindeer across all cooperatives for the period 2004-2015 showed a trend of more frequent condemnation at late slaughter dates (**Fig. 1)**. The proportion of liver condemnation peaked during December in the southern region and March in the central and northern regions (**Fig. 1)**.

Liver condemnation among slaughtered reindeer varied from 0-100% in different years and in different cooperatives (**Fig. 2)**. The mean proportion of liver condemnation in a given year differed between herds sharing the same grazing area, herds being slaughtered on the same day and between the same herds being slaughtered on different days.

We found geographical variation in liver condemnation, with a strong tendency for clustering and a similar proportion of condemned livers in neighboring cooperatives each year. This created hot spots consisting of several cooperatives with spatially clustered outbreaks. Interestingly, the clusters appeared to move around Lapland from year to year. None of the outbreaks affected all of Lapland in any of the investigated years. More outbreaks were recorded in central Lapland (around Sodankylä) in 2004-2007, whereas more outbreaks were recorded in southern Lapland (around Kuusamo) after 2010. Only a small number of outbreaks were recorded in the northern part of Lapland during 2004-2015. Overall, outbreaks were more frequent in the southern part of the reindeer-herding area compared to central or northern Lapland (**Fig. 2)**.

There was a large variation in the daily proportion of liver condemnation among different cooperatives in the same area. Some cooperatives showed high liver condemnation in one batch (animals slaughtered on a specific day), but the following batch (within 4-5 weeks from the first) could show a low proportion of liver condemnation. For example, in the southern region, the proportion of liver condemnation on 18th November 2004 for cooperative number 42 was 61% (n = 57; 95% CI: 45.6-77.2), while 4 weeks later on 15th December, the proportion was significantly lower ($p<0.05$) at 29% (n=135; 95% CI: 24.7-34.5). While cooperative 51 had a high proportion of liver condemnation (52-71%) in 2004, a nearby cooperative (48) with no organs condemned in the six slaughter batches in the same year (**Fig. S3**).

Model results:

***The proportion of liver condemnation in reindeer vs. predicted transmitted L3 from an infectious reindeer*:**

We used the model to estimate the cumulative number of L3 transmitted per week per infectious reindeer, based on both meteorological and microclimatic temperatures. We also identified the maximum proportion of liver condemnation in each week among the cooperatives in all three regions. A comparison between the predicted cumulative number of L3 transmitted and the maximum proportion of liver condemnation per week in the three different regions is presented in **Fig. 3**.

A higher proportion of liver condemnation was detected in the southern and central regions than in the northern region for most of the year. Liver condemnation began earlier (September) in the southern region compared to central and northern regions (October). The model predicted that worms mature to cause liver damage at around week 40-42, the same time that liver condemnation also starts to increase in different cooperatives. The models (for both meteorological and microclimatic temperatures) also showed a plateau in the potential number of transmitted mature worms at the same time that the maximum proportion of liver condemnation was reached. The model predicted that worm transmission from vector to host starts and ends within a very short period of time (6-8 weeks) between mid July and late August. Any vectors biting infectious reindeer outside this period will be infected, but the worms will not be able to mature or be transferred to a new host. This period during which reindeer were infected was shorter than the development time from L3 to new microfilaria, making it impossible for *S. tundra* to have more than one generation each summer. Therefore, all new infections transmitted from mosquitoes to reindeer in one summer originated from reindeer infected the previous summer.

For some years, the model based on meteorological temperature predicted that L3 transmission was not possible, and the model based on microclimatic temperatures predicted it was possible. For example, in 2007 in the southern region, >40% liver condemnation was observed in slaughtered reindeer, but our FMI-based model suggested that L3 transmission would not be possible. However, the microclimatic temperature-based model predicted that L3 transmission would be likely in some years (e.g. 2009) when liver condemnation was low.

Likewise, the FHI-based model did not predict any potential transmission in 2012, but the microclimatic model predicted moderate L3 transmission.

Overall, the model based on FMI temperature predicted a higher number of L3 potentially transmitted in the years when the proportion of liver condemnation was high (**Fig. 4**). When there was more than 20% liver condemnation in at least two slaughter batches, our model predicted a higher potential L3 transmission in 8 out of 10 years (80%) in the southern region and 6 out of 7 years (86%) in the central region 1 out of 4 years (25%) in northern region. When less than 5% of livers were condemned, our model showed no/low worm transmission in 1 out of 2 years in the southern region, 3 out of 5 years in the central region and 6 out of 7 years in the northern region (**Fig. 4**). Across the three regions, our model predicted a higher potential worm burden in 15 out of 21 occurrences (sensitivity: 71%) when a higher proportion of livers were condemned. Likewise, the model predicted no/very low transmission in 11 of the 16 occurrences (specificity: 73%) when there was no/low liver condemnation.

In all three regions, the model based on estimated microclimatic temperature predicted a higher potential L3 transmission in 18 of 22 occurences (sensitivity: 81%) and predicted no/very low transmission in 8 of the 14 occurrences (specificity: 57%) when there was no/low liver condemnation (**Fig. 4**).

The duration of the effective transmission period from host to vector (model output):

The duration of the effective transmission period and the potential cumulative L3 transmitted from an infectious reindeer are depicted in **Fig. 5**. The mean duration of the annual effective transmission period was 15.5 days (range: 2-28 days) in the southern region, 17.1 days (range: 4-29 days) in the central region and 14 days (range: 9-30) in the northern region. The M-K test identified a trend of increasing duration over the years (p-value <0.01 for both southern and central regions; tau values of 0.38 for the southern region and 0.36 for the central region). The trend was not significant for the northern region.

The mean (10-90th percentiles) potential number of L3 transmitted each year was on average 1,741 (0-2,648) in the southern region, 1,538 (0-1,869) in the central region, and 154 (range: 0-889) in the northern region. The M-K test identified an increasing trend in the potential number of L3 transmitted over the years (p-value <0.01 for the southern region and central region; tau values of 0.36 for the southern region and 0.32 for the central region). The trend was not significant for the northern region.

The longest transmission period was predicted for 2013 in the south, 2005 in the central region and 2014 in the north. The highest number of L3 were transmitted in 2003 in the southern and central regions, and 2004 in the northern region. The correlation coefficient between the duration of an effective transmission period and the potential worm burden was 0.35 in the southern region, 0.37 in the central region and 0.48 in the northern region (**Fig. 5**).

The duration of the effective transmission season of *S. tundra* from reindeer to mosquitoes estimated from the FMI-temperature-driven model is presented in **Fig. S4**. Only mosquitoes that received infected blood meals with *S. tundra* microfilaria between the first week of June and last week of July were able to survive long enough for worms to develop into infective stage L3 and transmit *S. tundra* to a new susceptible reindeer.

Discussion

There was wide variation in the proportion of liver condemnation between different cooperatives in the same region (south, central or north), and within different herds in the same cooperative. There was also wide variation in liver condemnation among the animals slaughtered in the same week within the same cooperatives. This indicates there may be some local factors affecting the transmission of *S. tundra* in reindeer. The variation may be a result of the difference in mosquito abundance between different herds in the same cooperative, the coverage of anthelmintic administration by the different owners, or the density of reservoir animals (moose, roe

deer, forest wild reindeer), but may also be related to differences in observations made by meat inspectors. The efficiency of the meat inspector greatly influences liver condemnation. The meat inspection findings were made by several veterinarians. All the reindeer meat inspectors have participated in education for meat inspection and harmonizing meat inspection decisions, and the reporting. Because minor changes are not included in the meat inspection decisions, the true incidence of these indicators is likely higher [18]. In other studies, slaughterhouse observations signaled large variation in the proportion of liver condemnation due to cysticercosis in pigs in France [29]. There are two general causes of misclassification based on meat inspection: the inspector might 1) miss minor/microscopic lesions (poor sensitivity); 2) classify lesions due to diseases other than *S. tundra* (poor specificity).

On average, the proportion of liver condemnation due to *S. tundra* infection in reindeer increased gradually over time until December in the southern region and March in the central and northern regions. Liver condemnation occurs due to adult worms present in the reindeer [6]. The reindeer were infected with the L3 stage of *S. tundra* between June and August, and after 2-4 months when these larvae become adults, they can cause pathogenesis. An inspector can therefore only detect changes in organs after 2.5-3 months of the reindeer being infected – after the majority of worms have developed into adults. The reason that the proportion of liver condemnation decreases again after December (in the southern region) or March (in the central and northern regions) is not understood, but may be related to clinical recovery [30]. The older reindeer discarded as breeding stock are slaughtered during this period, and might have a lower infestation due to immunity acquired from cumulative exposure to *S. tundra*. However, the available data did not allow us to distinguish the age group of slaughtered animals.

Our model predicted the potential transmission of L3 over the year and was not developed to predict the proportion of liver condemnation. Estimated L3 transmission is the potential number of worms transmitted from an infectious reindeer. Whether an outbreak leading to liver condemnation will occur depends on the actual

abundance of infectious reindeer present in the area. If a large number of infectious reindeer are present to infect the vectors, susceptible reindeer will receive more L3 worms and hence be more likely to have organs condemned in the autumn. The actual abundance of vectors was not available for the particular years in this study, but is likely to vary and is expected to affect the accumulated transmission. Our model assumed infectious reindeer to have a specific daily density of microfilaria that would be the same for all years and regions, which may not be the case. Therefore, the number of worms originating from one reindeer as predicted by the model is not directly comparable to the proportion of liver condemnation.

We compared the two independent datasets of the predicted accumulated L3 transmitted per infectious reindeer and the observed condemnation. We expected them to correlate, though not perfectly because: i) the data were not equivalent (proportion of *S. tundra* infection in slaughtered animals vs. predicted accumulated number of L3 worms transmitted); ii) the data could not be expected to be linearly correlated (proportion of liver condemnation is 0-100% and potential L3 transmission is unlimited); iii) we do not know how many worms must be present before condemnation will occur; iv) there is a potential human bias when identifying the pathology caused by *S. tundra* in different slaughterhouses; v) we do not know how many reindeer are actually infected at the start of the season as this depends on last year's transmission, the deworming treatment and slaughter rates during winter. It is therefore not possible to estimate how many L3 are transmitted to the population, only how many L3 are transmitted from an infectious reindeer. Despite these limitations, the model could predict the occurrence of outbreaks fairly well (sensitivity: 71-81%; specificity: 57-73%).

Our model suggested higher transmission rates in the southern region, with transmission starting earlier and lasting longer than in the northern region. This correlated very well with field observations of the proportion of liver condemnation. Furthermore, the model detected a positive trend in the duration of the transmission period and potential worm burden in the southern and central regions, which corresponds very well to slaughterhouse findings. Overall, our predictions of potential worm burden each season matched fairly well with the observed weekly maximum proportion of liver condemnation in reindeer (except in the northern region).

However, our model based on FMI temperature did not predict outbreaks in some of the years when outbreaks occurred. For example, in 2007, our model showed transmission would not be possible in the southern region, but inspectors at the slaughterhouses detected a large outbreak. However, when using microclimatic temperature to drive the model, a high L3 transmission was predicted for that year. These findings indicate that microclimatic temperature at sites where vectors might rest while digesting blood meals may play an important role in the local transmission of *S. tundra* in reindeer, as has been found for other vector-borne infections [12,17,31]. Microclimatic temperature is considered to be very important for other ecological phenomenon in Finland due to its landscape of boreal forest, lakes and bogs [32,33].

Our model showed that there is only one transmission cycle possible in any year within the reindeer-herding areas in Lapland, and transmission is therefore entirely dependent on some reindeer with microfilaria escaping the deworming treatment during the winter period. It is not possible to have more than one transmission generation per year in Finland as the L3 larvae stage requires a long time (90-120 days) to develop into adult worms in the reindeer and produce microfilaria. This means one infected reindeer at the beginning of the season might infect many susceptible reindeer, but none of the newly infected reindeer would be able to transmit the infection to a new host within the same year. Therefore, the model suggests it is possible to prevent outbreaks by deworming all infected reindeer and other host such as roe deer, moose, during winter. This is an important finding that could facilitate decision making in terms of the control and prevention of *S. tundra* in Finnish reindeer. Around 80% (range: 64-90%) of reindeer are currently dewormed with the anthelmintic drug ivermectin during the autumn round-up (September to November) [30]. This allows for at least 20% of the animals to act as carriers of the parasite. Untreated animals could cause an outbreak if a suitable summer season were to follow, but the outbreaks could not increase exponentially via several generations of transmission (as for diseases with a much shorter incubation time, e.g. bluetongue virus). For *S. tundra* infection in reindeer, there is a linear relationship between the proportion of animals that escape deworming during winter and the number of L3 worms transmitted to susceptible animals.

Our model identified an increasing trend for both the duration of the effective transmission season as well as the potential worm burden for the period 1979-2015 for the southern and central regions. This finding agrees with the proportion of liver condemnation observed during 2004-2015, when there were a number of large outbreaks recorded in these areas. However, only a small number of outbreaks were identified in the northern region during this period, and our model showed a non-significant trend in the northernmost regions of Lapland. Approximately 30-40% of southern and central regions have wetland and bogs rich in water suitable for mosquito breeding, whereas bog habitats make up only around 12% of the northern region[34]. Due to the geographical location, this northern region is generally much colder than the other studied areas (see annual mean summer temperature in Finland: **Fig. S5**). Furthermore, in northern areas there are more open and windy spaces, and snowy mountains which is expected to allowing reindeer to escape vectors thus potentially resulting in lower biting rates [32]. This could result in lower vector abundance and thus cause only small outbreaks in the northern region, while the model calculations assumed identical vector densities across all three regions. This may explain why our model showed a poor correlation between predicted worm burden and the proportion of liver condemnation in slaughtered reindeer in the northern region.

Temperature is an important parameter for vector-borne disease transmission [35]. There was a low proportion of liver condemnation in 2008 in the southern region. Our meteorological temperature model predicted no transmission in 2008 as the temperature was not high enough to allow worms to fully develop in the vector (EIP). In a situation like this, the abundance of mosquitoes will have no impact on model output (i.e. whatever the size of the mosquito population, the model will have no accumulated transmission at the end of the season). Our model assumed the same mosquito densities across all three regions and years (2004-2015). However, this would not be the case in reality, and could lead to misleading results. Our model also shows that an outbreak is only possible if the mosquitoes have the infection early in the season (early May to mid-July) so they can survive long enough to complete the EIP and bite a susceptible host. However, the extent of the outbreak will depend on the timing of the peak period of mosquito abundance, microfilarial density and

490 temperature. The outbreak in 2014 was the result of such a combination; temperatures peaked in mid-July when
491 mosquito abundance was also high, along with high microfilaria densities in their blood. Warm summers alone
492 therefore may not be enough for a large *S. tundra* outbreak in Finland, as the timing of warm periods, high
493 mosquito abundance and microfilaria density also plays a vital role. The poor-to-moderate correlation between
494 the duration of an effective transmission period and the potential number of L3 transmitted (0.35-0.48; both
495 estimated from the model) also implies that the length of season alone is not enough to generate a large outbreak.
496 If mosquitoes were infected very late in the season, the pathogen development would be slow (due to lower
497 temperatures) and it might not be possible to complete the EIP within its lifespan. Our study therefore shows that
498 temperature plays a crucial role in the transmission of *S. tundra*.
499
500 Our model predicted higher worm transmission in 71-81% of cases with a high proportion of organ
501 condemnation, and lower or no transmission in 57-73% of cases with low/no liver condemnation. This means
502 that our model correlated with the true outbreaks very well, but did not correlate well for years with no
503 outbreaks. A possible reason for this could be that *S. tundra* in Finland can transmit at lower temperatures than
504 other filaraoid nematodes (14°C) [14]. Our model could not capture this because the equation we used was not
505 specific to *S. tundra* and the temperature-dependent development rate of *S. tundra* might be different from other
506 filarioid nematodes. Another possible reason could be that microclimatic temperatures are higher than we
507 estimated.
508
509 This study did not use actual recordings of vector abundance, microfilaria densities, nor mathematical
510 formulae specifically developed according to the development time of *S. tundra*. The vector abundance used in
511 our model was based on recordings from one season at one location. There are no published data on mosquito
512 life history (biting rate, survival rate, life span etc.) available from Finland, and the formulae we used for the
513 biting rate of mosquitoes were based on studies using laboratory conditions in tropical countries (Thailand and
514 Puerto Rica). The mathematical calculation of EIP used in this study was originally developed from *Dirofilaria*

immitis. We developed an independent biological process-based model using parameter estimates from the literature, rather than a statistical model fitted to the outbreak data [4]. The model is therefore independent of the condemnation data, and it is interesting that the predicted L3 transmission from one infectious reindeer correlates well with the liver condemnation data. We assumed that all microfilaria ingested by a mosquito would become L3 if the mosquito survived long enough, and that all L3 larvae would be transmitted to a new host. In reality, only a fraction of the microfilaria in a mosquito will become L3, and only a small fraction of these will be transmitted to a new host.

Conclusions

The proportion of liver condemnation increased as the date of slaughter advanced. There was a geographical variation in the proportion of liver condemnation across different reindeer-herding areas and in different years. We found the effective transmission period for *S. tundra* to be very short for reindeer in Lapland, but it showed an increasing trend over the period. Only one generation of transmission was possible in each season, indicating the possibility of controlling *S. tundra* outbreaks in Finland by deworming all animals effectively at the end of summer. Temperature was an important driver of *S. tundra* outbreaks in Finnish reindeer. It is possible that warm summers alone are not sufficient; rather the timing of warm periods, high mosquito abundance and microfilaria density plays a vital role in determining the extent of outbreaks, as our model predicted a very short transmission window. The model predicted higher worm transmission when a larger proportion of organs were condemned (sensitivity: 71-81 %), and lower or no transmission when low/no liver condemnation was recorded (specificity: 57-73%). Local factors including microclimatic temperature, vector abundance, microfilaria density and anthelmintic coverage might play an important role in determining the extent of outbreaks and these should be investigated in more detail.

Abbreviations

FMI: Finish Meteorological Institution

EIP: Extrinsic Incubation Period

M-K test: Mann-Kendall test

Declarations

Acknowledgments:

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Availability of data and materials:

The *S. tundra* datasets in this study are not publicly available as they include confidential production data on reindeer diseases from reindeer-herding cooperatives and slaughterhouses. This is based on the decision of Regional State Administrative Agencies of Lapland no. LAAVI/171/03.02.01/2015. The meteorological data can be obtained from FMI upon reasonable request.

562 **Authors' contributions:**

563 NH led the collaboration among DTU, FMI, EVIRA, MAZAMA, collected and analyzed the data and wrote the
564 manuscript. SL provided the slaughterhouse data on *S. tundra*, provided technical assistance in understanding
565 reindeer husbandry, interpreted the findings and arranged NHs field visit to Finnish reindeer-herding areas. LJK
566 and AO provided critical input in manuscript writing. RB planned the original study and critically reviewed the
567 manuscript. All authors read and approved the final version of the manuscript.

568

569 **Ethics approval:** Not applicable.

570 **Consent for publication**: Not applicable.

571 **Competing interests**: The authors declare that they have no competing interests.

572

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672 **Figure legends:**

673 **Fig 1:** Seasonal variation in the proportion of liver condemnation due to *S. tundra* infection in reindeer.
674 Information was retrieved through slaughterhouse data during meat inspection in different weeks (top) and
675 months (bottom), 2004-2015. Only a small number of records were available during weeks 52, 53 and 1.

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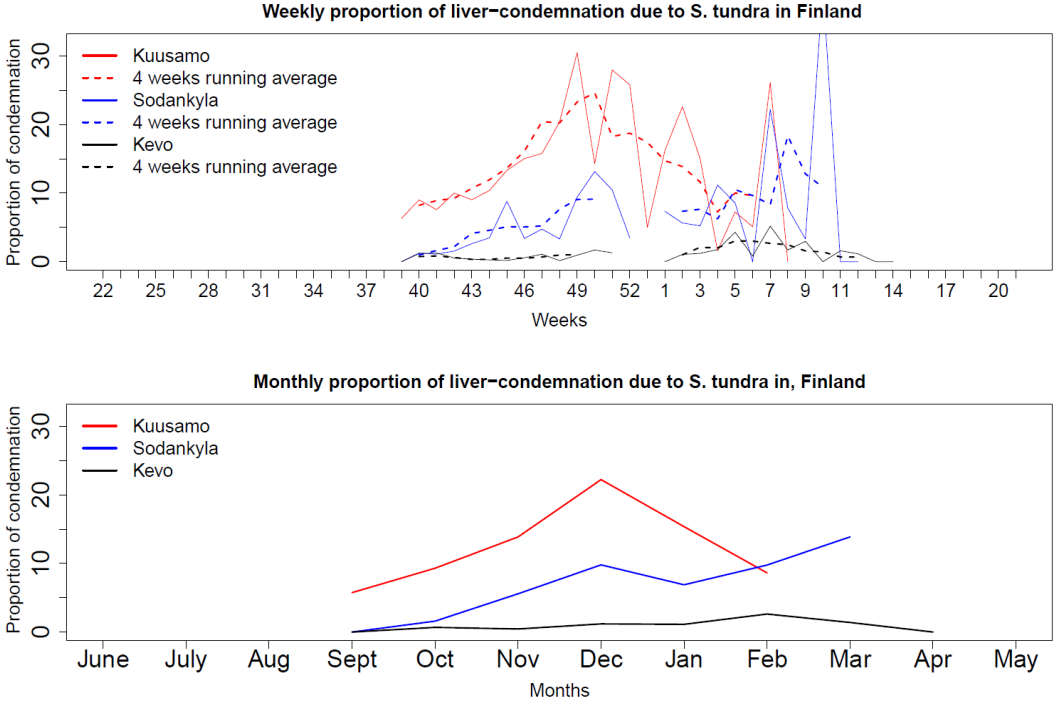

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Fig 2: Map of Lapland showing the proportion of liver condemnation due to *S. tundra* infection based on slaughterhouse inspection data from reindeer cooperatives. The year 2004 here indicates the period between 1st June 2004 and 31st May 2005. Outbreaks in different cooperatives were grouped together, but there were no fixed foci of outbreaks.

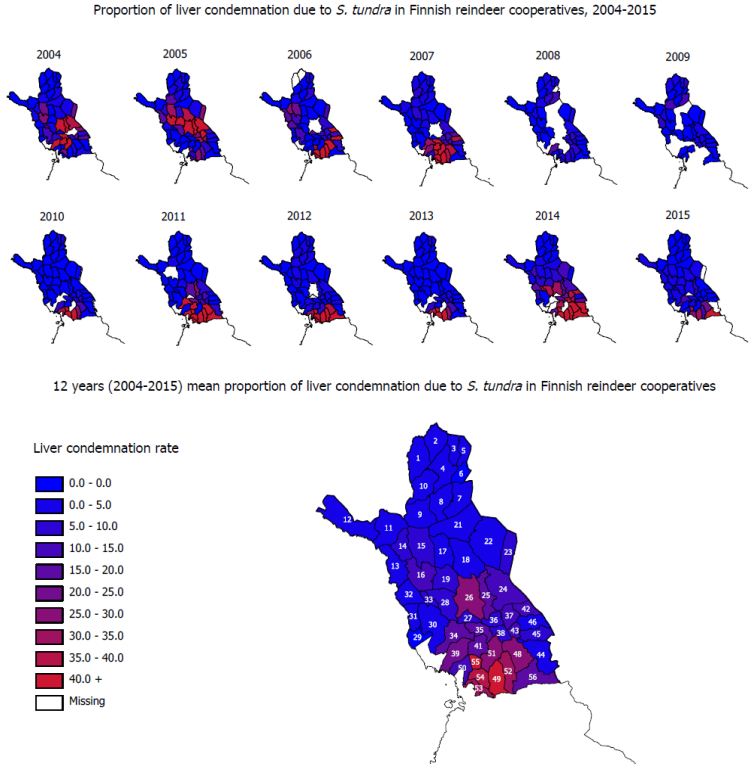


Fig 3: Potential cumulative number of *S. tundra larvae* L3 transmitted from one infectious reindeer and the maximum proportion of liver condemnation due to *S. tundra* in Finnish reindeer per week (based on slaughterhouse observations) in the southern region, Kuusamo (red), the central region, Sodankylä (blue) and the northern region, Kevo (black) of Lapland, 2004-2015. Note the two Y-axes have different units. The model based on Finnish meteorological temperatures did not detect worm transmission during the years 2007, 2008 and 2015 in Kuusamo (southern region), whereas the model using estimated microclimatic temperatures showed moderate-to-high worm transmission in those years. Liver condemnation and predicted potential worm burden peaked during the same period.

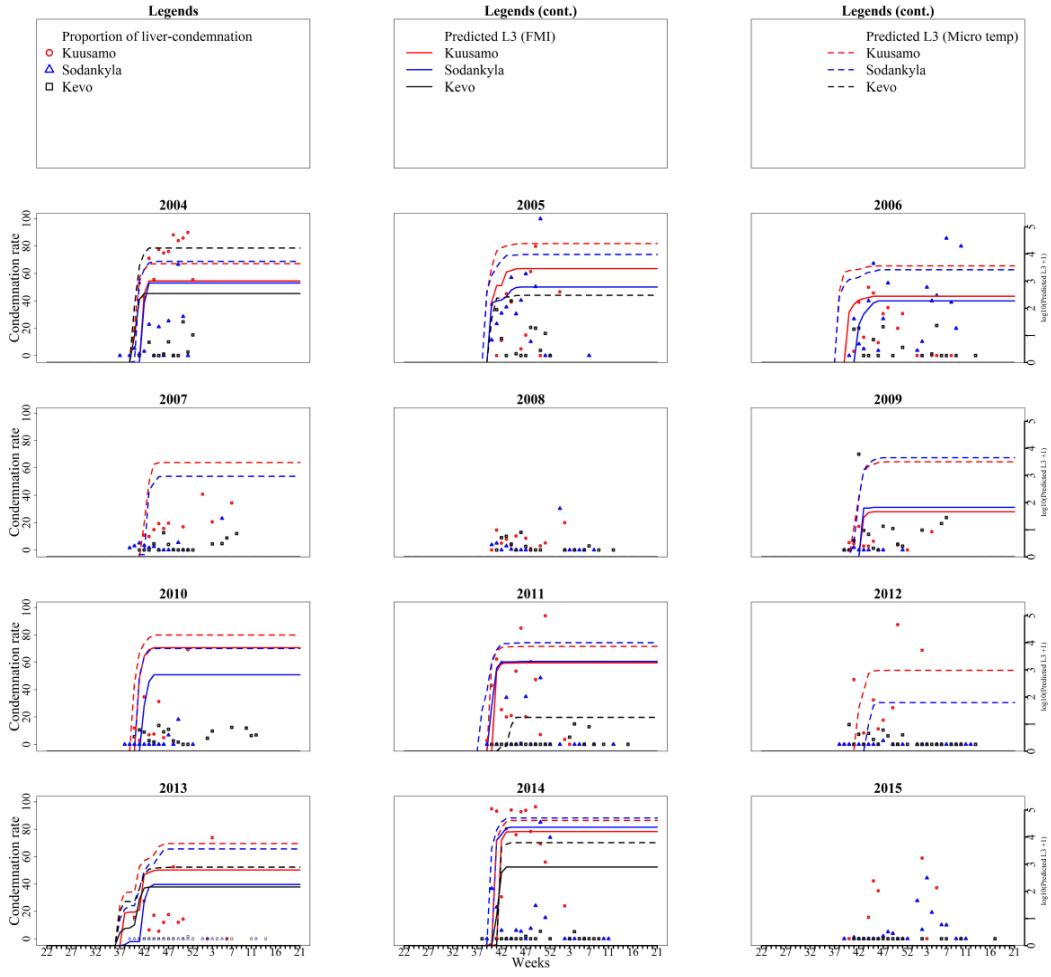


Fig 4: A comparison between the weekly maximum proportion of liver condemnation among the cooperatives in three different regions and the predicted potential transmission of L3 (predicted worm) from an infectious reindeer in the respective cooperatives. The vertical dotted black line (>20%) indicates the threshold for identifying the year with a higher proportion of worm transmission. Note the two Y-axes have different units.The model driven by FMI temperature showed a higher number of L3 being transmitted in 8 of the 10 years (80%) in the southern region, 6 of the 7 years (86%) in the central region and 1 of the 4 years (25%) in the northern region, coinciding with a high proportion of liver condemnation. The model driven by estimated microclimatic temperature showed a higher number of L3 being transmitted in 9 of the 10 years (90%) in the southern region, 6 of the 7 years (86%) in the central region and 2 of the 4 years (50%) in the northern region, coinciding with a high proportion of liver condemnation.

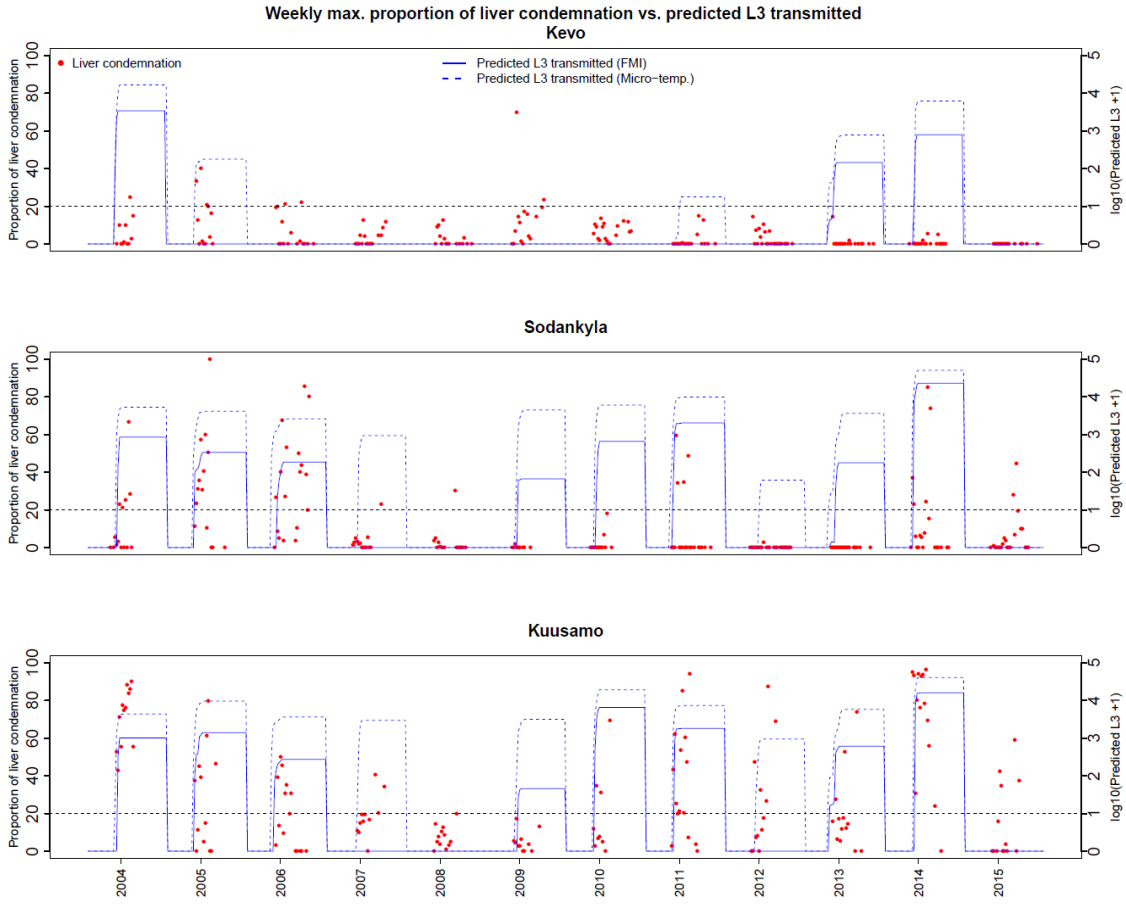
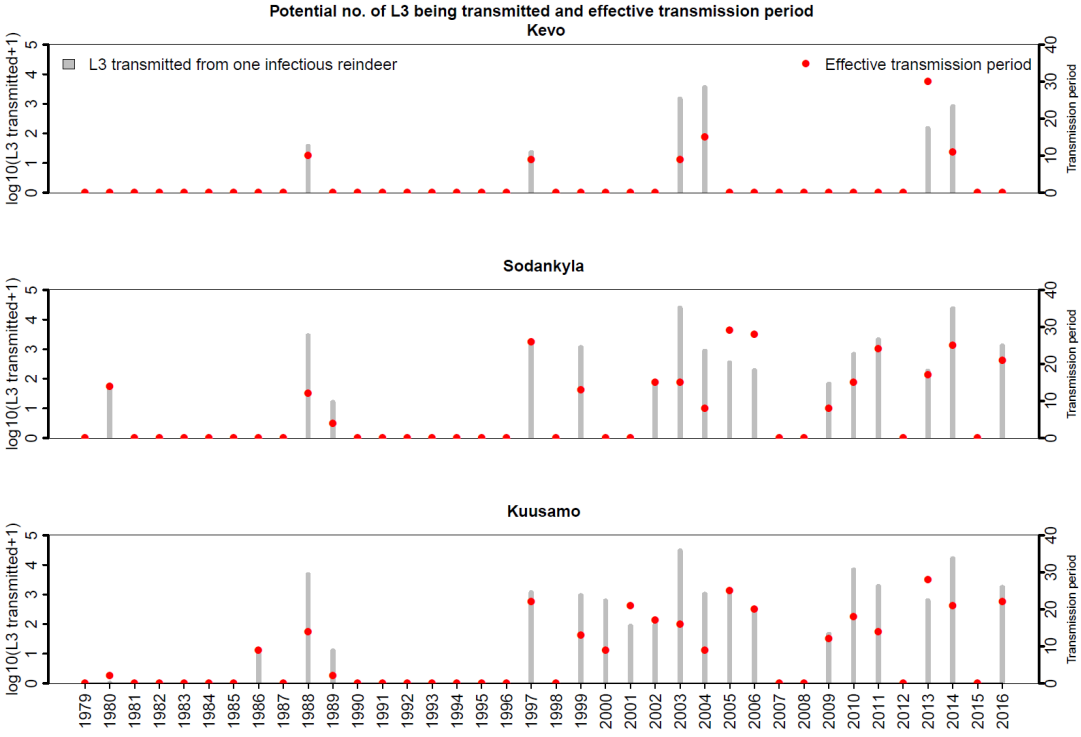


Fig 5: The cumulative number of *S. tundra larvae* L3 transmitted from one infectious reindeer at the end of a season (gray bars). The red dots indicate the duration (in days) of the annual transmission season when mosquitoes receive infected blood meals and survive to successfully transmit the L3 stage of *S. tundra* to a reindeer. Note the two Y-axes have different units.



Table

Table 1: The equations used to model changes in the Extrinsic Incubation Period (EIP) of *S. tundra* in mosquitoes, blood meal digestion period (biting rate) and daily survival rate of mosquitoes

Traits	Equations	References
EIP of *S. tundra* in mosquitoes	1/((T-14)/130)	[12,14] [15]
Blood meal digestion rate (biting rate)	1/(0.0943 + 0.0043*T)	[36]
Survival rate of mosquitoes	$e^{-1/(-4.4 + 1.31*Tmean - 0.03*(Tmean)^2)}$ Daily maximum survival is set as 90%	[23,37]
T = Temperature (hourly), Tmean = Daily Mean temperature		

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Supplementary files (Manuscript –IV): Figures (S1-S5)

The annual, temporal and spatial pattern of *Setaria tundra* outbreaks in Finnish reindeer: a mechanistic transmission model approach

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Fig S1: Flow diagram of the *S. tundra* lifecycle in reindeer and mosquitoes

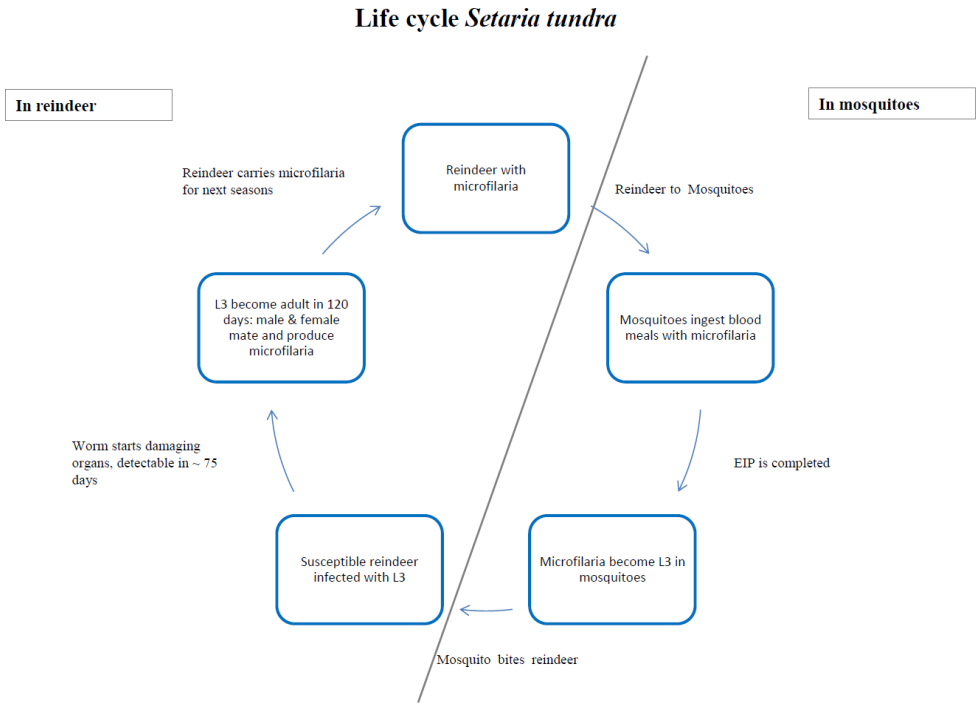


Fig S2: The daily abundance of mosquitoes (ref), and density of microfilaria per ml of blood from reindeer in Oulu Zoo (ref) in 2004, used for the *S. tundra* transmission model.

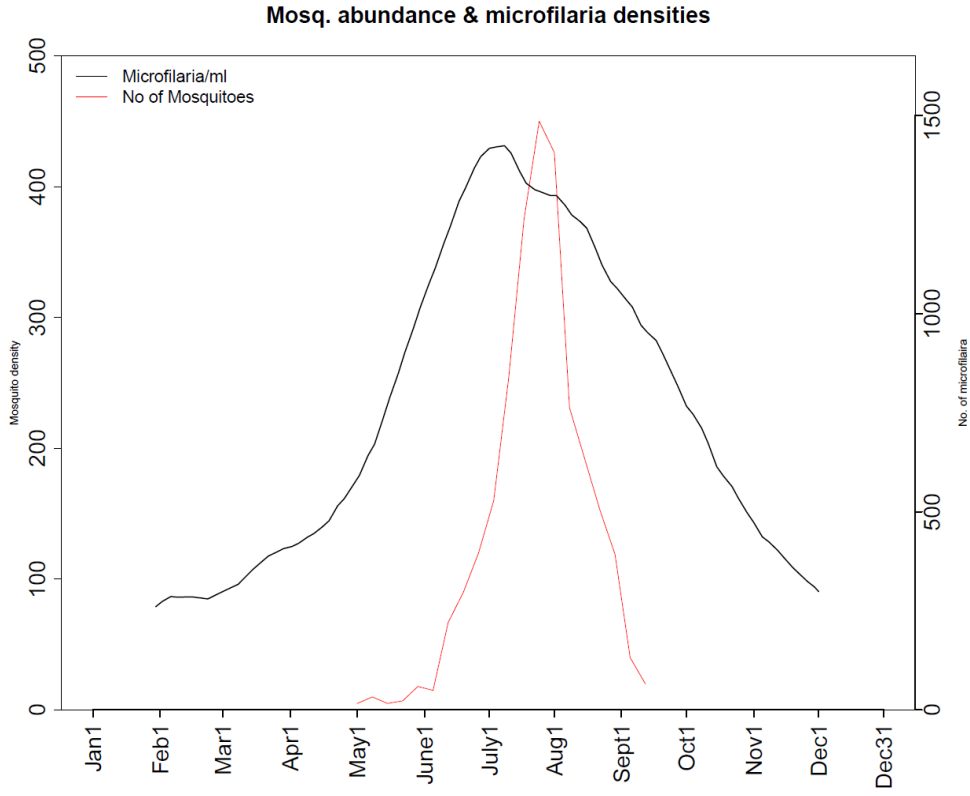


Fig S3: The proportion of liver condemnation across different cooperatives in the southern region, Kuusamo (circles), central region, Sodankylä (triangles) and northern region, Kevo (rectangles) from 2004-2015.

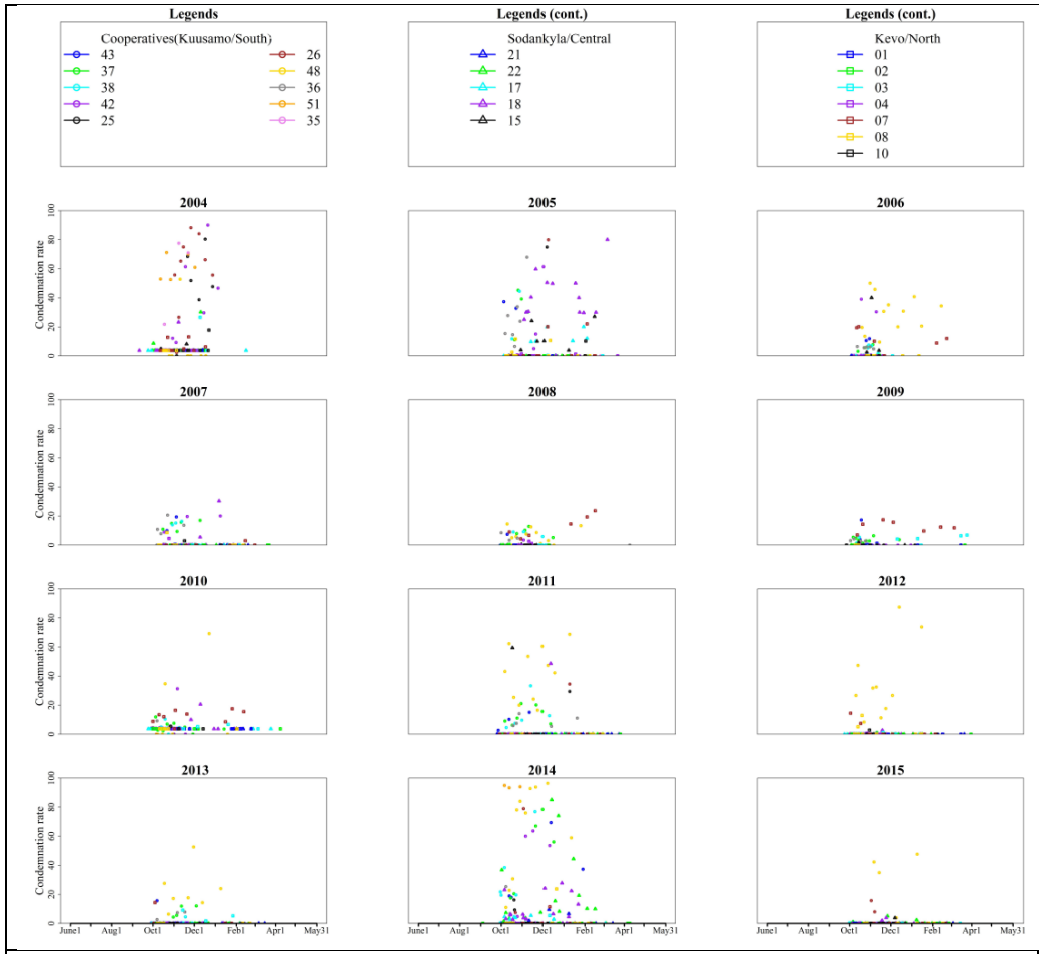


Fig S4: The estimated period when *S. tundra* microfilaria can be transmitted from reindeer to mosquito vectors. Only microfilaria that were successful in becoming L3 *S. tundra* from the same vector is shown. The estimated microfilaria transmitted from one infectious reindeer at three locations in Lapland: northern (Kevo), central (Sodankylä) and southern (Kuusamo) are presented by the date when the mosquitoes received the microfilaria infected blood meal. The dates the reindeer are infected with L3 cannot be seen from this graph. Central and southern regions had the longest duration of transmission period and the largest number of microfilaria transmitted in 2014 (cyan, dotted), whereas transmission for the northern region (Kevo) peaked in 2004 (blue).

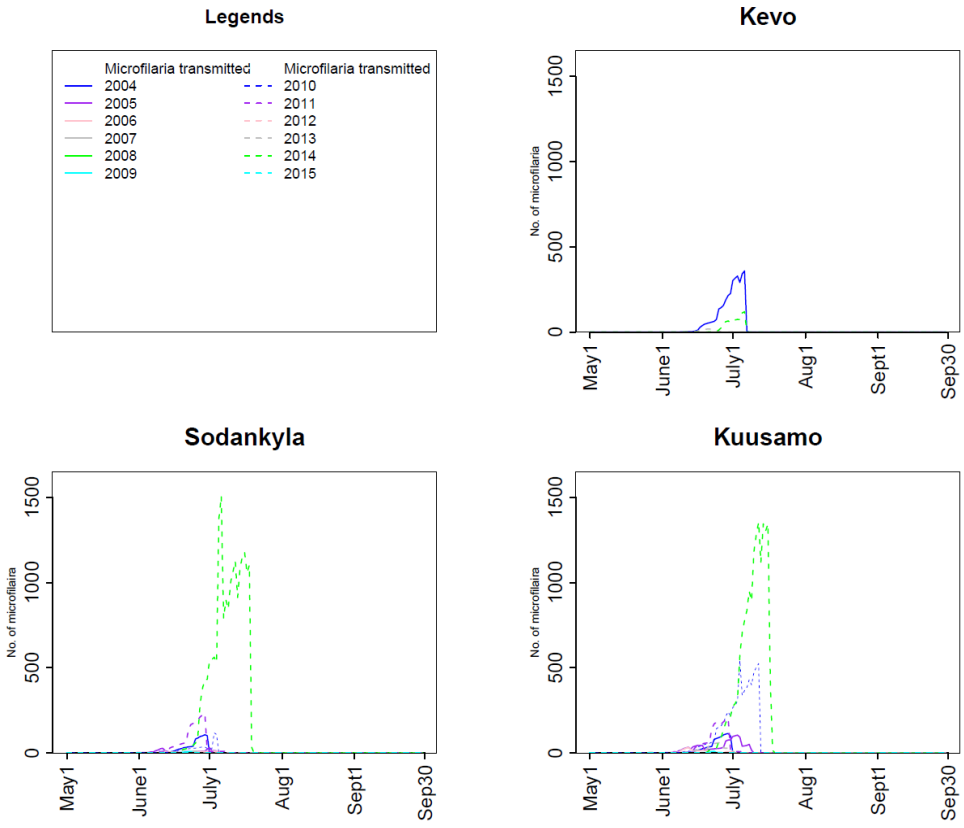
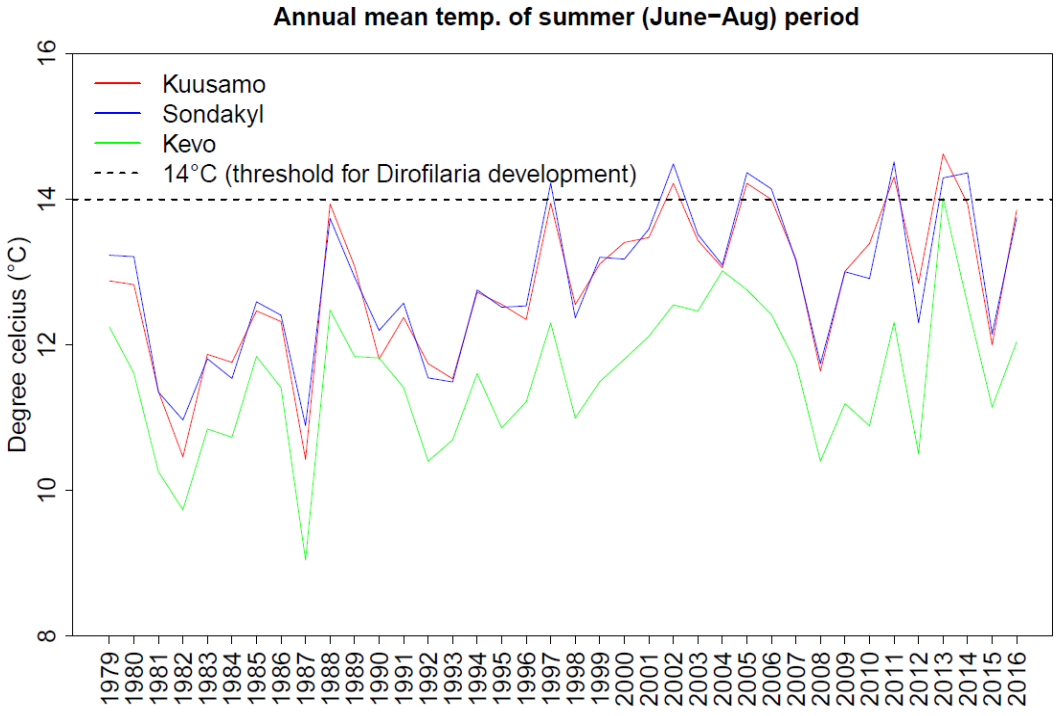


Fig S5: The annual mean temperature for the summer period (June-August) in the southern (Kuusamo), central (Sondakyla), and northern (Kevo) regions of Lapland.



Chapter 4: Discussion

4. Discussion

The primary aim of this thesis was to improve our current understanding of the transmission parameters of VBD in northern Europe. Temperature is one of the key drivers of VBD transmission models. We conducted four studies with different meteorological and microclimatic temperatures, and showed that microclimatic temperature increases the potential for vector-borne disease transmission by shortening the duration of the pathogen development period, increasing the duration of the transmission season and extending transmission to geographical areas previously considered unsuitable for VBD transmission. We studied the uncertainty/variations associated with the parameters of BTV transmission and found a large uncertainty/variation for the equations used to estimate the daily insect survival rate and the transmission rate of BTV from host to vector.

4.1 Difference between meteorological and microclimatic temperature

The microclimatic temperature in five out of six habitats (dry meadow, hedges, forest, cattle grazing field, horse grazing field) was generally warmer than the meteorological temperature reported by the DMI for the same area. However, there were relatively small differences in the average monthly mean temperature between DMI records and the different microclimatic habitats (for example 1.0-1.3°C in dry meadow in May 2015). However, the differences between the hourly DMI temperatures and microclimatic temperatures were more distinct, as microclimatic temperatures were (3.5-5°C) warmer during the day and (1-3°C) cooler at night. Dry meadow was the warmest and this is the most abundant habitat found around Danish cattle farms. Wet meadow was the coolest habitat. Temperatures recorded at different heights of microclimatic habitat varied across the seasons. This is due to the variation in vegetation as it grows, which can prevent sunlight from reaching the lower parts and may reduce the wind speed. During the beginning of the warm season (spring), the lower areas of the habitats were warmer than the upper and mid-height areas, whereas during the warm season (late summer and autumn), the temperature at lower areas became cooler as the vegetation grew and blocked the sunlight. This shows that microclimatic temperatures are complex, and variation in temperature depends on local factors such as seasonal vegetation growth.

4.2 Modeling microclimatic temperature

Standard weather station temperatures are available from national meteorological institutes for any site in Europe, but hourly microclimatic temperatures are not. We developed a linear regression model using meteorological parameters (hourly temperature, solar radiation, humidity, and wind speed) to predict the microclimatic temperature of six different potential vector habitats, resulting in a R^2 of 0.87-0.96.

The key parameters that determined the microclimatic temperature were: hourly temperature, solar radiation, humidity, wind speed, and the temperature recorded in the previous hour. The temperature during the previous hour was a significant variable in the model, indicating that it takes time for the temperature in a microclimatic habitat to change. For example, if solar radiation heats up the vegetation and soil in the habitat, it will take time to cool down, while the standard meteorological temperature 2 m above ground can change much more rapidly. We recommend using this prediction model to estimate the microclimatic temperature of potential insect habitats in other settings with climates similar to that in Denmark. However, if climatic parameters (hourly temperature, solar radiation, wind speed and humidity) are not available, we suggest adding 3.5 to 5°C to the daily maximum and deducting 1 to 3°C from the daily minimum temperature and then using a standard method (e.g. sinusoidal distribution [105]) to convert these into hourly temperatures. We followed this procedure to estimate the microclimatic temperature of northern Finland and used the estimated microclimatic temperature in a mechanistic transmission model for *Setaria tundra*. The model based on estimated microclimatic temperature had better sensitivity when identifying years with a higher liver condemnation rate due to *S. tundra* in reindeer compared to the model based on standard meteorological temperature (86% vs. 80%). However, the model based on microclimatic temperature had a lower specificity compared to the model based on meteorological temperature (61% vs. 75%; Manuscript IV).

4.3 Impact of microclimatic temperature on vector-borne disease transmission

We found three important differences when comparing VBD transmission models using microclimatic temperatures versus meteorological temperatures: 1) transmission models based on microclimatic temperature had a shorter virus development period, 2) transmission models based on microclimatic temperature had a longer transmission season, and 3) transmission models based on microclimatic temperature resulted in a transmission range expansion in geographical areas considered unsuitable for potential VBD transmission using models with meteorological temperatures.

4.3.1 Shortening of the virus development period:

Virus development or completion of the extrinsic incubation period (EIP) is a key step in VBD transmission. In temperate regions of the world, virus development in insects is slow due to low environmental temperatures. The normal lifespan of mosquitoes or *Culicoides* (30-60 days) is often not long enough to accomplish the EIP and support transmission of a viral disease. We used both microclimatic and meteorological temperatures to estimate the EIP of six pathogens (e.g. bluetongue, Schmallenberg virus, and West Nile virus, Dirofilaria, dengue and malaria). In five out of six habitats, the EIP was shorter when estimates based on microclimatic temperature (rather than meteorological temperature) were used.

The microclimatic temperature was warmer during the day and cooler at night than the DMI temperature. During the spring (April-May), the night-time DMI and microclimatic temperatures were both below the temperature threshold that halts virus development. There were many hours in summer (June-August) when the estimates for both microclimatic and meteorological temperature were above the threshold, but the microclimatic temperature was much higher than the DMI temperature. In general, viruses develop at an exponentially increasing rate at increasing temperatures above the threshold until, for some pathogens, an upper threshold (e.g. 34.4°C for Malaria [36]) is reached. The higher daytime temperature in microclimatic habitats therefore had a relatively large impact on the EIP (Manuscript I). For example, for Schmallenberg virus, development was possible in an average of 24.4 days with DMI temperatures, but just 13.3 days with cattle grazing field temperatures, 13.4 days with horse grazing field temperatures, 15.2 days with dry meadow temperatures, 15.7 days with hedge temperatures, 24.1 days with forest/tree temperatures and 25.5 days with wet meadow temperatures [Manuscript I] [38].

Our findings are in agreement with a study from Chennai, where microclimatic temperature contributed to a shortened EIP for both vivax and falciparum malarial parasites [36].

4.3.2 Increased transmission season duration

Knowing the duration of transmission seasons is important for policy making in order to control and prevent VBD. We found that the duration of the VBD transmission season increased when microclimatic temperature was used in the model. For example, when using the meteorological temperature for 2015, Schmallenberg virus development was not possible after mid-August. However, when the observed microclimatic temperature was used in the same EIP equation, virus development was possible for an additional month [Manuscript I].

Most BTV cases were identified in late autumn (between September and October) during the 2008 BTV outbreak in Denmark, with the last case detected on November 17, 2008 [13]. Although the actual date of infection could have been earlier due to a delay in identifying the outbreaks, it is still surprising that BTV can be transmitted at such a low temperature in Denmark. Our model using microclimatic temperature showed that the last date when a cohort of vectors could be infected with BTV and later be able to infect new hosts upon completion of the EIP was in the second week of September (early autumn). Considering the maximum lifespan of *Culicoides* is 60 days, successful transmission would even have been possible in mid-November (late-autumn) in Denmark [Manuscript III].

When using the estimated microclimatic temperature, our *S. tundra* transmission model showed a longer duration of the effective transmission season in three regions for the period 1979-2015 compared to when temperatures recorded by the Finnish Meteorological Institute (FMI) were used. For example, in the southern (Kuusamo) region, the mean duration of the effective transmission season estimated by the FMI temperature model was just 15.5 days, compared to 27.0 days using estimated microclimatic temperature. In the central (Sodankyla) region, the mean duration of the effective transmission season was 17.0 days using the FMI model and 25.5 days using the estimated microclimatic temperature model. In the northern (Kevo) region, the mean duration of the effective transmission season was 14.0 days using the FMI model and 17.6 days using the estimated microclimatic temperature model.

4.3.3 Geographical expansion:

We found a spatial pattern of farms with shorter EIP for Schmallenberg virus, which tended to be grouped together in the southern part of Denmark. However, we found that some farms located in northern Denmark (Jutland) also had a very short EIP for Schmallenberg virus. The farms located in the far north were surrounded by warmer microclimatic habitats (dry meadow) and thus might have an equal risk of Schmallenberg virus outbreaks compared to farms with cooler microclimatic habitats, as was seen in the south (Zealand; Manuscript II). These findings show that even countries covering small geographical areas like Denmark may need to implement strategies to identify, control and prevent VBD based on how rapidly a virus can develop within different regions.

In the *S. tundra* study, the transmission model based on FMI temperature showed that out of the total 38 years, the temperature was suitable for EIP completion in 19 years in the southern (Kuusamo) region, 17 years in the central (Sodankyla) region, and 6 years in the northern region (Kevo). When we used the estimated microclimatic temperature, the EIP completed in most of the years in the central and southern regions (33 out of 38 years) and more than half of the years (20 of 38) in the northern region [Manuscript IV]. Therefore, Kevo (the northernmost location in Finland) had the potential for *S. tundra* transmission in half of the studied years. In reality, *S. tundra* has frequently been detected in Finnish reindeer-herding areas since 2003 [11], and high rates of organ condemnation due to *S. tundra* have been recorded most years since 2004 in at least one reindeer-herding cooperative in the three regions [106].

4.4 The microclimatic temperature of Danish cattle farms and Schmallenberg virus transmission potential

We generated one of the largest microclimatic temperature datasets in Europe for 22,004 cattle farms for each hour over 17 years (2000-2016). These data will be useful in the future for modeling diseases that are sensitive to temperature, including VBD.

We only modeled one temperature for a geographical area using the DMI, but found that 62% of cattle farms in Denmark were surrounded by more than one type of vegetation and

were therefore likely to have more than one microclimate. We predicted four different microclimatic temperatures (dry meadow, hedges, wet meadow and forest) at each farm and for each hour.

We did not expect to find a large variation in temperature around Danish cattle farms as it is a small country of only 42,931 km^2. However, we found considerable differences in the microclimatic temperature of habitats within a 500m radius of the farms. The difference between the warmest and coolest microhabitats at the same farm was on average 3.7°C (5^{th} and 95^{th} percentiles: 1.0°C to 7.8°C). These differences resulted in a four-fold increase in Schmallenberg EIP between the coolest and the warmest microclimates. Such a large difference highlights the importance of microclimatic temperature variation and the need to incorporate this into temperature-sensitive disease models.

4.5 Bluetongue virus transmission potential in Denmark

BTV has caused disease outbreaks in two consecutive years (2007 and 2008) in Denmark [13]. The prediction of high daily IB in our model is therefore not a surprising finding. The worst-case scenario estimated the daily mean (maximum) IB at 449 (2,899), whereas the best-case scenario estimated transmission was not possible in Denmark. The mean number of daily IB was 2.2. Considering the long incubation period of BTV, IB >2 for the entire season indicates that bluetongue disease can spread rapidly if introduced to a farm, especially since a host remains infectious for approximately 3 weeks [96]. The effective transmission period from host to vector lasts almost 3 months and starts in the third week of May, ending in the third week of August. The highest number of IB was estimated in the month of July with daily mean of 6.3 IB.

4.6 Key drivers of the BTV transmission model

Temperature plays a critical role in driving many BTV transmission parameters including the EIP, *Culicoides* survival rate, *Culicoides* biting rate and host-to-vector transmission rate. These parameters are all correlated. For example, at a higher temperature, the EIP will be shorter and the biting rate will be faster but the daily survival rate of the insects will be lower. In the BTV model, we used the minimum temperature each hour to estimate the IB with BTV, which predicted transmission, was not possible on any day in the best-case

scenario. There was no transmission because virus development was not completed within the lifespan of the vectors. In a situation like, this the number of insects is irrelevant; BTV transmission is not possible even with a high abundance of vectors.

However, when there are favorable temperatures, vector abundance plays an important role. The average number of IB estimated from the fourth quantile mean number of Obsoletus ensemble was 17 times higher than that estimated from the third quantile mean number of Obsoletus ensemble, and 408 times higher than estimates from the second quantile mean number of Obsoletus ensemble. This shows that temperature is very important for VBD transmission, and vector abundance plays a vital role when there are favourable temperatures because the number of vectors can vary substantially, while the variation in temperature is relatively small.

4.7 Variation in parameter estimates of infectious bites

Although the prediction of high numbers of potential IB with BTV was expected, the large variation associated with estimates of the total daily number of IB was surprising. While the model estimated a large number of IB with combinations of some equations, it also estimated low or no transmission with combinations of other equations. For example, the mean number of IB estimated by the survival rate equation suggested by Wittmann et al. [33] was 3.5 times higher than the equation suggested by Gerry & Mullens [42], and 6.1 times higher than survival rate equation suggested by Gerry & Mullens (second equation) [42]. On July 1, the mean number of IB varied from 0.01 to 11.1 (10-90th percentile).

We found the largest variation was associated with equations for insect survival rate and host-to-vector transmission rate. These are both very basic details required for *Culicoides*-borne disease modeling, and more studies are needed to establish the relationship between temperature, insect survival and transmission of BTV from host to vector.

4.8 Global warming and VBD outbreaks

Many studies have been conducted over the years on the potential impact of climate change and VBD outbreaks [10,82,83,93,98]. These studies suggested that VBD outbreaks will extend to geographical areas in which there are currently fewer outbreaks, and endemic countries will experience a higher number of outbreaks [10,82,83,93,98]. This PhD explored two events that are

related to potential climate change and VBD. In our study [Manuscript II], we found that the type of available resting sites and vector behavior [101] would largely determine how climate change could affect VBD in the future.

Insect vectors spend 90% of their lifetime resting while digesting their blood meals and producing eggs [12,77]. Our study showed that in Denmark, the difference between the warmest and coolest resting sites at the same time and on the same farm was on average 3.7°C (5th and 95th percentiles: 1.0°C to 7.8°C) [101]. If the insect vectors continuously look for cooler microhabitats, and if such microhabitats remain available, the predicted global warming of 0.2°C per decade [107] might not have a large impact on VBD transmission in the near future. However, if insects choose habitats randomly, the impact will be similar to what the model on the impact of climate change and VBD[82,108] currently predicts. If the insects choose warm habitats to rest on (especially to digest blood meals), the impact could be more severe than currently predicted [58,82,84,109]. Our study therefore identified the need to examine the resting behavior of insects in more detail.

For *Setaria tundra* outbreaks in Finland (Manuscript IV), our model identified an increasing trend for both the duration of the predicted effective transmission season, as well as the predicted potential number of larvae (L3) transmitted for the period 1979-2015 for the southern and central regions. These findings are in agreement with the observed proportion of liver condemnation due to *S. tundra* observed over the period 2004-2015, when there was a number of large outbreaks recorded in these areas.

4.9 Modeling aspects of Setaria tundra in Finnish reindeer

S. tundra is a mosquito-borne filarial nematode that causes peritonitis, perihepatitis and is responsible for poor body condition in reindeer [11]. Northern Finland (Lapland) experiences a very short warm season of 2-3 months, yet reindeer in Lapland still become infected with *S. tundra.* Vector-borne filarial disease has a different lifecycle to vector-borne viral diseases (e.g. bluetongue), in which all bites are considered infectious after the EIP is completed as the virus replicates in the vector, (although the virus load is not quantified). In filarial diseases, mosquitoes can only transmit a fixed number of L3 worms that they have ingested (in the microfilaria stage) while taking a blood meal from an infectious host, because microfilaria do not replicate in the mosquito. An adult male

and female worm must then mate in the final host in order to complete the sexual lifecycle and produce their offspring, the microfilaria [9]. Furthermore, on average 80% of reindeer are treated each year with the anthelmintic drug Ivermectin, which reduces the risk of worm survival in the host [110]. However, *S. tundra* outbreaks are commonly identified in the Finnish reindeer-herding area. We developed a mechanistic model to understand the transmission of *S. tundra* in Finnish Lapland.

Our model showed that temperatures never allowed for transmission of more than a single generation of *S. tundra* during each season in Lapland. The effective transmission period of *S. tundra* from reindeer to vector is very short, but it follows an increasing trend over the period in southern and central parts of Lapland. Increasing temperatures facilitate a range expansion and an increasing duration of effective transmission period for *S. tundra*.

To our knowledge, this is the first mechanistic transmission model developed to increase our understanding of the transmission dynamics of *S. tundra* in northern Europe. We believe the findings of this study will benefit the authorities and reindeer herders in arctic regions around the world when planning prevention strategies, and contribute to the understanding of how global warming may affect VBD in cold climates.

There were several difficulties in modeling *S. tundra* in Finland

1) Weekly mosquito abundance data was not available for an entire season in Lapland or any country of the same latitude
2) Laboratory experimental data on the developmental rate of *S. tundra* were not available (although an experiment was conducted at two fixed temperatures [11], but this was not enough to establish a relationship between temperature and *S. tundra* development)
3) Seasonal microfilaria density data were not available
4) Only daily minimum, maximum and mean temperature data were available for the period before 2000 and we therefore used this information in the analysis for consistency. Hourly temperature data was made available at FMI from 2000 onwards.

We have taken the following steps to manage these difficulties:

1) We used unpublished mosquito abundance data collected from Kuusamo in the year 2004. We assumed that mosquito abundance would remain at a similar level to what was observed in 2004 at all three sites, regardless of any annual variation in temperature. This may not be the case, but these were the most relevant mosquito abundance data available for use in the model.
2) We used the EIP equation for *Dirofilaria spp* [38,109]. This makes biological sense as the relationship shown at two fixed temperatures for the *S. tundra* development rate matches the well-established relationship between temperature and the *Dirofilaria spp*. development rate [11]. However, Setaria and Dirofilaria are two different organisms and *S. tundra* is endemic in the arctic climate.
3) We used microfilaria density data published from a 2004 study involving the natural infection of reindeer with *S. tundra* [97].

4.10 What is lacking in VBD modeling?

We conducted four different studies and conclude that there are some basic data still lacking in VBD modeling.

1) Basic studies on insect resting behavior and resting site preferences are very rare. Some studies were conducted in the 1930s and 1950s [79,111], but many of them contain little quantitative information and were mostly descriptive, with no systematic approach. Surprisingly, we simply do not know to this day where the insects rest, for how long, and what can be considered the optimal insect habitat.

2) We found large discrepancies between different parameter estimates for VBD, and it is likely that some of the estimates do not capture the real impact of temperature on VBD transmission parameters (daily insect survival rate, EIP, insect biting rate, host-to-vector transmission). The variation we identified for BTV transmission was large. For some parameter combinations, our model predicted a high number of daily IB, whereas for others we found a low/no possibility of transmission in Denmark. It is not possible for both of these predictions to be true, but unfortunately there are few equations available for estimating these parameters. The equations used here are also widely used by other modelers for estimating the R_0 of BTV.

3) A lack of data: *S. tundra* is a neglected disease for which parameter estimate data are not readily available. Epidemiologists usually face this kind of problem when modeling a relatively new disease, and although *S. tundra* is not a new pathogen, we faced similar problems. However, our model, which is based on mimicking biological processes, could explain the outbreak data very well. The model predicted worm transmission for different regions over different years. The model showed a plateau in the predicted number of worms during the same time when the maximum proportion of liver condemnation was reached.

4.11 What is new?

This PhD research can potentially add to the existing knowledge on VBD with the following:

1) *Systematic study of microclimatic temperatures in insect habitats*:
We used a systematic approach to record the microclimatic temperature of six different insect habitats. In each habitat, we studied microclimatic temperature at 2 or 3 different heights for an entire warm season (May to October) at two different locations in Denmark. We recorded the hourly microclimatic temperature data with 66 temperature loggers.

2) *Systematic study on the impact of microclimatic and meteorological temperature on VBD transmission*:
We studied the impact of microclimatic temperature on the EIP of six pathogens (Manuscript I), the spatial and temporal variation in the transmission potential of Schmallenberg virus in Denmark (Manuscript III), and the transmission potential of BTV in Denmark (Manuscript III). In addition, we used estimated microclimatic temperatures to predict *S. tundra* L3 transmission in reindeer in Finnish Lapland (Manuscript IV).

3) *Models for microclimatic temperature*: We developed a predictive model to estimate the microclimatic temperature of six different microclimatic habitats at three different heights (Manuscript I). The model that used meteorological

parameters could explain 87-96% of the variation observed in the microclimatic habitats. The model could be useful in habitats with a similar climate and vegetation as those found in Denmark.

4) *Estimation of microclimatic temperature for habitats surrounding 22,004 cattle farms in Denmark*: This is one of the largest microclimatic temperature datasets for predicting disease transmission in Europe. This will allow researchers to utilize farm-level temperature data to model diseases that are sensitive to temperature (Manuscript II).

5) *Modeling S. tundra to understand the biological processes leading to transmission*: Previous statistical models have been fitted to *S. tundra* outbreak data from Finland using annual summer temperatures to establish a relationship between outbreaks and temperature and the potential effect of global warming [10]. We developed a mechanistic transmission model (fitted independently of the outbreak data) to explain *S. tundra* outbreaks in Finland.

6) *Parameters of the BTV transmission model*: We estimated a large variation in the number of daily IB, showing that some of the equations used for estimating BTV transmission parameters may not be relevant to the Danish climate and may not be practical to use in BTV modeling.

Chapter 5: Conclusions

5. Conclusions

This PhD project addresses practical questions for modeling VBD in Denmark and northern Europe. The specific conclusions from each study are listed in Chapter 3 (Manuscripts: I-IV). The main conclusions are listed below:

1. Microclimatic vs. meteorological temperature
    - Microclimatic temperature was warmer during the day and cooler during the night than the meteorological temperature
    - There were relatively small differences in the average monthly mean temperatures between DMI records and the different microclimatic habitats
    - Microclimatic temperature varied among different habitats, heights and seasons
    - Not all microclimatic habitats were warmer than the meteorological temperature (wet meadows were colder than the recorded temperature at the nearest weather station)
    - Dry meadow is the warmest habitat and it is also the most abundant habitat within a 500 m radius of the 22,004 cattle farms in Denmark
    - About 62% of cattle farms are surrounded by more than one potential insect habitat and these farms therefore have more than one microclimatic temperature
    - The warmest microclimatic habitat of a cattle farm is on average 3.7°C (5^{th} and 95^{th} percentiles: 1.0°C to 7.8°C) warmer than the coolest microclimatic habitat at the same time.

2. Impact of microclimatic and meteorological temperature:
   Compared to the model that used traditional meteorological temperatures, the microclimatic temperature increased the potential for VBD transmission by:
    - Shortening the virus development period / duration of the EIP
    - Increasing the duration of the transmission season
    - Extending the range of VBD transmission to geographical areas that were otherwise considered unsuitable for potential transmission

3. Model to predict microclimatic temperature:
   We developed a regression model able to predict the microclimatic temperature of four different potential insect habitats using only four parameters from

meteorological data (hourly temperature, solar radiation, wind speed and humidity), resulting in R^2 values ranging from 0.87 to 0.96. To our knowledge, this is the first published model for predicting the temperature of potential insect habitats.

4. Temporal and spatial variation in the transmission potential of Schmallenberg virus:

- Farms with a shorter EIP for Schmallenberg virus were grouped together in the southern part of the country (comprising southern Funen and associated islands, Lolland, Falster, and southern Zealand)
- There were also groups of farms with a shorter EIP located in the far north (Jutland)
- The completion of virus development depends on the temperature in the actual microclimatic habitats in which the insects rest. This emphasizes the need to know more about insect behavior and resting site preferences.

5. BTV transmission potential in Denmark

- The mean (10-90th percentiles) number of IB estimated for BTV was 2.2 (0-8.9) per day for the entire transmission period (April-October)
- The worst-case scenario estimated a transmission season of 6 months (mid-April to mid-October), with a maximum daily number of IB of 2,899
- The best-case scenario in our model showed that no transmission of BTV was possible
- Temperature and vector abundance were the two most influential drivers of BTV transmission
- We identified a large variation in the estimated number of IB associated with equations for the different parameters
- The equation used for the daily insect survival rate and the host-to-vector transmission rate resulted in a large variation in the daily number of estimated IB.

6. *S. tundra* outbreaks in Finnish reindeer

- Our model showed that temperatures never allowed for the transmission of more than a single generation of *S. tundra* in one season
- Our model predicted an increasing trend during the period 1979-2015 for both the duration of the effective transmission period of *S. tundra* and for the

potential number of larval stage 3 *S. tundra* being transmitted from an infectious reindeer

- Warm summers alone are not sufficient to cause *S. tundra* outbreaks, but the timing of warm periods, high mosquito abundance and microfilaria density all play a vital role in determining the extent of outbreaks as our model predicted a very short transmission window
- Local factors including microclimatic temperature, vector abundance, microfilaria density and anthelmintic coverage might also play an important role in determining the extent of outbreaks.

Chapter 6: Perspectives

6. Perspectives

There are still many areas relating to vectors and VBD that need to be investigated.

One basic study that is required would answer the question of where the insects rest, for how long, and what factors influence their choice of resting sites. I was asked these questions throughout my PhD at different conferences, during presentations and in formal and informal discussions. This would be primary research and the field would benefit greatly from knowing more about the resting behavior and resting site preferences of vectors in order to predict VBD transmission more accurately.

If I am able to continue my research, I will study VBD outbreaks and their potential association with climate change. My hypothesis is that insects will react differently in selecting resting sites at different temperatures. If they do not actively select resting sites based on temperature, we may expect an increase in VBD as global warming takes effect. If insects do have a preference for certain temperatures when selecting resting sites, we may be able to control VBD transmission. If the insects prefer cooler microhabitats for resting, it is likely that an increase in temperature of 0.2-0.4 degrees per decade [107] will not greatly affect VBD transmission, as cooler microhabitats will still be available. However, if they prefer warmer sites, we may have to control for the warmer resting habitats by increasing the amount of habitat with cooler microclimatic temperatures (e.g. forest and wet meadow). This may be difficult to implement, but would be beneficial in the long term.

Practical research for Denmark:
We developed a dataset to perform a risk assessment of VBD in Denmark. I suggest the following practical research for Denmark:

1. Risk assessment for important livestock diseases: African horse sickness virus, epizootic hemorrhagic diseases, bovine ephemeral fever, lumpy skin disease and equine encephalosis
2. Risk assessment for zoonotic/human diseases – West Nile Virus, Dirofilariasis, Usutu virus, dengue, Zika and malaria, Japanese Encephalitis virus , Oropouche virus

In the future, we might be able to use more relevant data (microclimatic temperature, insect abundance) and more accurate parameters for modeling VBD.

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